U.S. DEPARTMENT OF COMMERCE
National Technical Information Service
PB-297 926

Ambient Water Quality Criteria: Polynuclear Aromatic Hydrocarbons

(U.S.) Environmental Protection Agency, Washington, DC

1978

PB 297926

Polynuclear Aromatic Hydrocarbons

Ambient Water Quality Criteria

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U. S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

Criteria and Standards Division
Office of Water Planning and Standards
U.S. Environmental Protection Agency
Washington, D.C.

BIBLIOGRAPHIC INFORMATION

PB-297 926

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1978

PERFORMER: Environmental Protection Agency, Washington, DC.
Criteria and Standards Div.

Section 304(a) of the Clean Water Act (33 U.S.C. 1314(a)), requires EPA to publish and periodically update water quality criteria. These criteria are to reflect the latest scientific knowledge on the identifiable effects of pollutants on public health and welfare, aquatic life, and recreation. This report presents water quality criteria for polynuclear aromatic hydrocarbons. It presents concentration criteria for the protection of fresh water and saltwater aquatic life. It presents 'safe' concentrations for humans, and in the case of suspect or proven carcinogens, gives various levels of incremental cancer risk. A section 304(a) water quality criterion is a qualitative or quantitative estimate of the concentration of a water constituent or pollutant in ambient waters which, when not exceeded, will ensure a water quality sufficient to protect a specified water use. Under the Act a criterion is a scientific entity, based solely on data and scientific judgment. It does not reflect considerations of economic or technological feasibility nor is it a water quality stand, ...

KEYWORDS: *Water quality, *Toxicology, *Aromatic polycyclic hydrocarbons, *Toxic substances, *Water pollution effects(Humans).

Available from the National Technical Information Service, Springfield, Va. 22161

PRICE CODE: PC A09/MF A01

CRITERION DOCUMENT

POLYNUCLEAR AROMATIC HYDROCARBONS

CRITERIA

Aquatic Life

For freshwater aquatic life, no criterion for any polynuclear aromatic hydrocarbon can be derived using the Guidelines, and there are insufficient data to estimate a criterion using other procedures.

For saltwater aquatic life, no criterion for any polynuclear aromatic hydrocarbon can be derived using the Guidelines, and there are insufficient data to estimate a criterion using other procedures.

Human Health

For the maximum protection of human health from the potential carcinogenic effects of exposure to polynuclear aromatic hydrocarbons (PAH) through ingestion of water and contaminated aquatic organisms, the ambient water concentration is zero. Concentrations of PAH estimated to result in additional lifetime cancer risks ranging from no additional risk to an additional risk of 1 in 100,000 are presented in the Criterion Formulation section of this document. The Agency is considering setting criteria at an interim target risk level in the range of 10^{-5}, 10^{-6}, or 10^{-7} with corresponding criteria of 9.7 ng/l, 0.97 ng/l and 0.097 ng/l, respectively.

Introduction

Polynuclear aromatic hydrocarbons (PAH) are a diverse class of compounds consisting of substituted and unsubstituted polycyclic and heterocyclic aromatic rings. PAH are formed as a result of incomplete combustion of organic compounds with insufficient oxygen. This leads to the formation of C-H free radicals which can polymerize to form various PAH. Among these PAH are compounds such as benzo[a]pyrene and benz[a]anthracene, which are well-known for their ubiquitous presence in nature and carcinogenic effects in experimental animals.

Under the Consent Decree in NRDC v. Train maximum permissible concentration are to be recommended for the following PAH: benzopyrene; benzanthracene; chrysenes; benzofluorantheses; indenopyrenes. In this report, criteria are recommended for PAH as a class, derived using available data concerning several of the most extensively studied individual carcinogenic components in the class. There are no published studies available which adequately compare the carcinogenic activities of all ten of the specified PAH under similar experimental conditions. Likewise, there are no data available concerning human responses to individual compounds in the PAH class, since environmental exposures to PAH invariably involve contact with complex, and usually undefined, PAH mixtures.

This report considers the various human health aspects associated with exposure to environmental levels of PAH. Particular attention is directed at the contribution of food, water, and air to the total human PAH exposure. Assessment of anticipated health risks is directed specifically at the development of PAH-induced cancers as being the endpoint of greatest concern.

AQUATIC LIFE TOXICOLOGY*

FRESHWATER ORGANISMS

Introduction

No standard toxicity tests have been reported for freshwater organisms and any polynuclear aromatic hydrocarbon (PAH) not discussed in documents on specific compounds (e.g., fluoranthene and acenaphthene). There are some data for bioconcentration during tests with model ecosystems for short periods of time.

Residues

No measured steady-state bioconcentration factors (BCFs) are available for acenaphthylene, anthracene, benzo[a]pyrene, 3-methylcholanthene, and phenanthrene; bioconcentration factors can be estimated using the octanol-water partition coefficients of 5,500, 28,000, 1,150,000, 9,300,000, and 28,000, respectively. These coefficients are used to derive estimated BCFs of 410, 1,400, 24,000, 120,000, and 1,400 for acenaphthylene, anthracene, benzo[a]pyrene, 3-methylcholanthene, and phenanthrene for aquatic organisms that contain about 8 percent lipids. If it is known that the diet of the wildlife of concern contains a significantly different lipid content, appropriate adjustments in the estimated BCFs should be made.

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*The reader is referred to the Guidelines for Deriving Water Quality Criteria for the Protection of Aquatic Life [43 FR 21506 (May 18, 1978) and 43 FR 29028 (July 5, 1978)] in order to better understand the following discussion and recommendation. The following tables contain the appropriate data that were found in the literature, and at the bottom of each table are the calculations for deriving various measures of toxicity as described in the Guidelines.

Miscellaneous

Lu, et al. (1977) conducted studies with benzo[a]pyrene in a terrestrial-aquatic model ecosystem and observed bioconcentration factors after 3 days ranging from 930 for the mosquitofish to 134,248 for *Daphnia pulex* (Table 1). Bioconcentration factors for *Daphnia magna* and *Hexagenia* sp. for a shorter time were 200 to 3,500 (Table 1).

CRITERION FORMULATION

Freshwater-Aquatic Life

Summary of Available Data

No freshwater criterion can be derived for any polynuclear aromatic hydrocarbon using the Guidelines because no Final Chronic Value for either fish or invertebrate species or a good substitute for either value is available, and there are insufficient data to estimate a criterion using other procedures.

Table 1. Other freshwater data for polynuclear aromatic hydrocarbons

Organism	Test Duration	Effect	Result (ug/l)	Reference
		Benzo(a)pyrene		
Alga, *Oedogonium cardiacum*	3 days	Model ecosystem, bioconcentration factor = 5,258	-	Lu, et al. 1977
Cladoceran, *Daphnia pulex*	3 days	Model ecosystem, bioconcentration factor = 134,248	-	Lu, et al. 1977
Snail, *Physa* sp.	3 days	Model ecosystem, bioconcentration factor = 82,231	-	Lu, et al. 1977
Mosquito, *Culex pipiens quinquefasciatus*	3 days	Model ecosystem, bioconcentration factor = 11,536	-	Lu, et al. 1977
Mosquitofish, *Gambusia affinis*	3 days	Model ecosystem, bioconcentration factor = 930	-	Lu, et al. 1977
		Anthracene		
Protozoa, *Paramecium caudatum*	60 min	90% lethal photodynamic response	0.1	Epstein, 1963
Cladoceran, *Daphnia magna*	1 hr	Bioconcentration factor = ~200	-	Herbes, 1976
Cladoceran, *Daphnia magna*	~24 hrs	Bioconcentration factor = 760	-	Herbes & Risi, 1978
Mayfly, *Hexagenia* sp.	28 hrs	Bioconcentration factor = 3,500	-	Herbes, 1976
		Benzo-(a)-anthracene		
Bluegill, *Lepomis macrochrius*	6 mos	87% mortality	1,000	Brown, et al. 1975

SALTWATER ORGANISMS

Introduction

As was true for freshwater organisms, no standard toxicity tests with saltwater organisms have been conducted with any polynuclear aromatic hydrocarbon. There are a variety of data for bioconcentration during short exposures.

Residues

No measured steady-state bioconcentration factors (BCFs) are available for acenaphthylene, anthracene, benzo[a]pyrene, 3-methylcholanthene, and phenanthrene; bioconcentration factors can be estimated using the octanol-water partition coefficients of 5,500, 28,000, 1,150,000, 9,300,000, and 28,000, respectively. These coefficients are used to derive estimated BCFs of 410, 1,400, 24,000, 120,000, and 1,400 for acenaphthylene, anthracene, benzo[a]pyrene, 3-methylcholanthene, and phenanthrene, respectively for aquatic organisms that contain about 8 percent lipids. If it is known that the diet of the wildlife of concern contains a significantly different lipid content, appropriate adjustments in the estimated BCFs should be made.

Miscellaneous

The data (Table 2) on bioconcentration of polynuclear aromatic hydrocarbons are lower than those observed with freshwater organisms (Table 1) but may be due to the short exposure periods. A polychaete worm was exposed to various crude oil factions and 96-hour LC50 values were between 300 and 1,000 μg/l (Neff, et al. 1976a).

CRITERION FORMULATION

Saltwater-Aquatic Life

Summary of Available Data

No saltwater criterion can be derived for any polynuclear aromatic hydrocarbon using the Guidelines because no Final Chronic Value for either fish or invertebrate species or a good substitute for either value is available, and there are insufficient data to estimate a criterion using other procedures.

Table 2. Other marine data for polynuclear aromatic hydrocarbons

Organism	Test Duration	Effect	Result (ug/l)	Reference
		Benzo[a]pyrene		
Eastern oyster, Crassostrea virginica	14 days	Bioconcentration factor = 242	-	Couch, et al.,
Clam, Rangia cuneata	24 hrs	Bioconcentration factor = 8.66	-	Neff, et al. 1976a
Clam, Rangia cuneata	24 hrs	Bioconcentration factor = 236	-	Neff, et al. 1976b
		Chrysene		
Clam, Rangia cuneata	24 hrs	Bioconcentration factor = 8.2	-	Neff, et al. 1976a
		Benzo[a]pyrene Edible Tissue		
Mudsucker, Gillichthys mirabilis	96 hrs	Bioconcentration factor = .048	-	Lee, et al. 1972
Tidepool sculpin, Oliogocottus maculosus	1 hr	Bioconcentration factor = .13	-	Lee, et al. 1972
Sand dab, Citharichthys stigmacus	1 hr	Bioconcentration factor = .02	-	Lee, et al. 1972
		Crude oil extract (fluorene)		
Polychaete worm, Neanthes arenaceodentata	96 hrs	LC50	1,000	Neff, et al. 1976a
		Crude oil fraction (phenanthrene)		
Polychaete worm, Neanthes arenaceodentata	96 hrs	LC50	600	Neff, et al. 1976a
		Crude oil fraction (1-methylphenanthrene)		
Polychaete worm, Neanthes arenaceodentata	96 hrs	LC50	300	Neff, et al. 1976a

POLYNUCLEAR AROMATIC HYDROCARBONS

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Mammalian Toxicology and Human Health Effects

EXPOSURE

Ingestion from Water

The uptake of PAH in humans from water occurs through the consumption of drinking water. In the United States, the sources of drinking water are ground waters and surface waters, such as lakes and rivers. Although a small amount of PAH originates from natural or endogenous sources, the predominant sources of PAH in surface waters are man made. The discharges of raw and industrial sewage, atmospheric fallout and precipitation, road run off, and leaching from polluted soils, all of which contain substantial PAH concentrations (Andelman and Suess, 1970), contribute to the PAH contamination in surface waters. Other than leaching from soils, the only source of PAH in ground water is of endogenous origin. Borneff (1977) estimated that low-level contaminated river and lake waters contain five times higher PAH concentration than ground water, whereas in medium-level polluted river and lake waters this value may be 10 to 20 times higher. The concentration of PAH in ground water obtained by various authors is given in Table 1.

The PAH level in surface waters was determined by a number of German, English and Russian workers. In all of these methods, the PAH were solvent extracted from the water, subjected to cleanup procedures and analyzed either by TLC-spectrofluonmetry or by u.v.-spectrophotometry. These values are presented in Table 2.

TABLE 1

PAH Concentration in Ground Water

Source	Concentration, µg/l BaP	 Carcinogenic PAH	 Total PAH	Reference
G. Finthen, Germany,		0.002		Borneff, 1964
Mainz, Germany		0.005		Borneff, 1964
Unspecified locations in Germany	0.0004	0.003	0.04	Borneff & Kunte, 1964
Average of 12 German ground waters[a]			0.06	Borneff & Kunte, 1969
Champaign, Ill.[a]	N.D.[b]	0.003	0.007	Basu & Saxena, 1977-78
Elkhart, Ind.[a]	0.004	0.004	0.02	Basu & Saxena, 1977-78
Fairborn, O.[a]	0.0003	0.0008	0.003	Basu & Saxena, 1977-78

[a] These are results of 6 specified PAH

[b] N.D.: not detected

TABLE 2

Concentration of PAH in surface waters

	Concentration, µg/l			
Source	BaP	Carcinogenic PAH	Total PAH	Reference
Rhine River at Mainz	0.08	0.49	1.12	Borneff & Kunte, 1964
River Main at Seligentadt	0.0024	0.155	0.48	Borneff & Kunte, 1964
River Danube at Ulm	0.0006	0.067	0.24	Borneff & Kunte, 1964
River Gersprenz at Munster	0.0096	0.047	0.14	Borneff & Kunte, 1964
River Aach at Stockach	0.017	0.95	2.5	Borneff & Kunte, 1965
River Schussen	0.01	0.20	1.0	Borneff & Kunte, 1965
River Plyussa:				
at Shale-oil effluent discharge site	12			Dikun & Makhinenko, 1963
3500 m downstream	1			Dikun & Makhinenko, 1963
at Narvy water intake	0.1			Dikun & Makhinenko, 1963
A river:				
15 m below coke by-product discharge site	8-12			Fedorenko, 1964
500 m downstream	2-3			Fedorenko, 1964
Thames River				
at Kew bridge	0.13	0.18	0.50	Harrison, et al. 1975
at Albert bridge	0.16	0.27	0.69	Harrison, et al. 1975
at Tower bridge	0.35	0.56	1.33	Harrison, et al. 1975

Keegan (1971) analyzed the PAH content in three relatively unpolluted U.S. river waters by removing the PAH from water by solvent extraction. The extract was subjected to cleanup and the PAH were analyzed by TLC-spectrofluonmetry. Only samples from the Oyster River showed detectable amounts of four PAH. No PAH could be detected in the other two water samples from the Cocheco and Winnepesaukee Rivers.

The PAH levels in surface waters used as raw water sources for drinking water, and the effects of treatments of these waters on PAH levels, are shown in Table 3.

According to Borneff (1971), in surface waters, one-third of the total PAH is bound to larger suspended particles, a third is bound to finely dispersed particles, and the last third is present in dissolved form. The particle-bound portion of PAH can be removed by sedimentation, flocculation and filtration processes. The remaining one-third dissolved PAH usually requires oxidation for partial removal/transformation. The use of Cl_2, ClO_2, O_3, and U.V. light for this purpose has been studied. According to Borneff (1977), 50 to 60 percent of BaP can be removed by chlorination of water. However, the total PAH is reduced to a smaller degree by chlorination. ClO_2 on the other hand, reduces BaP concentration by 90 percent. But at BaP concentrations lower than 10 ppt, ClO_2 no longer functions as an oxidant for the transformation of BaP. The transformation of PAH is faster with O_3, but the use of O_3 requires intensified prepurification to prevent oxidation of other chemicals. Filtration with activated carbon has been suggested by Borneff

TABLE 3

Concentrations of PAH in Raw and Treated Surface Water used as Drinking Water Sources

Source	Treatment	Concentration, µg/l			Reference
		BaP	Carcinogenic PAH	Total PAH	
River Rhine	Untreated	0.082	0.485	1.11	Borneff & Kunte, 1964
River Rhine	Bank and activated carbon filtered	0.0005	0.015	0.13	Borneff & Kunte, 1964
Lake Constance	Untreated	0.0013	0.030	0.065	Borneff & Kunte, 1964
Lake Constance[a]	Rapid sand filtration Chlorination	0.0017	0.017	0.053	Borneff & Kunte, 1964
English River	Untreated	0.06[b]	0.37[c]	0.73[b]	Harrison, et al. 1976
English River	Filtration & Chlorination	0.009	0.051[c]	0.24	Harrison, et al. 1976
Monongahela River at Pittsburgh	Untreated	0.04	0.14	0.60	Basu & Saxena,1978
same as above	Treated[d]	0.0004	0.002	0.003	Basu & Saxena, 1978
Ohio River at Huntington, W. Va.	Untreated	0.006	0.020	0.058	Basu & Saxena, 1978
same as above	Treated[d]	0.0005	0.002	0.007	Basu & Saxena, 1978
Ohio River at Wheeling, W. Va.	Untreated	0.21	0.57	1.59	Basu & Saxena, 1977-78
same as above	Treated[d]	0.002	0.011	0.14	Basu & Saxena, 1977-78
Delawater River at Philadelphia	Untreated	0.04	0.16	0.35	Basu & Saxena, 1978
same as above	Treated[d]	0.0003	0.002	0.015	Basu & Saxena, 1978
Lake Winnebago at Appleton, Wis.	Untreated	0.0006	0.002	0.007	Basu & Saxena, 1977-78
same as above	Treated[d]	0.0004	0.002	0.006	Basu & Saxena, 1977-78

[a] These are average of five determinations with the exclusion of a sixth high value

[b] These values are estimates on the basis of average PAH adsorption in reservoir

[c] These values may be a little higher due to the inability of separation of all the carcinogenic from non-carcinogenic PAH

[d] The treatment included flocculation, activated carbon addition, filtration, pH control, chlorination and fluoridation.

(1977) as the best method for PAH removal/transformation during water treatment. The reduction of BaP concentration with activated carbon was 99 percent efficient in actual field tests (Borneff, 1977). With the exception of Appleton, Wis. drinking water, this finding of Borneff (1977) has been validated by the work of Basu and Saxena (1978, 1977-78), who demonstrated an 88 to 100 percent reduction of PAH in U.S. drinking waters by the use of activated carbon. In the case of Appleton, Wis. water, the initial PAH level in raw water was very low. Therefore, it can be concluded that below a certain minimum concentration, activated carbon may not be very effective for PAH removal/transformation.

As some derivatives of BaP and other PAH are formed during the disinfection of water with oxidizing agents and U.V. radiation, it is of interest to examine briefly the carcinogenicity of such derivatives. With the exception of alkylated derivatives, most BaP derivatives at best have only weak carcinogenic activity (Butenandt and Dannenberg, 1956). However, 10-chloro-compounds do cause tumors (Andelman and Suess, 1970). The quinones, some of which are also formed during chlorination (Andelman and Suess, 1970) do not produce tumors (Butenandt and Dannenberg, 1956), and may, in fact, inhibit the activity of other carcinogens (Buu-Hoi, 1959). The possibility of transformation of PAH into other carcinogenic compounds during water treatment processes is an area which remains largely unexplored.

The PAH content in U.S. drinking waters was analyzed by Basu and Saxena (1978, 1977-78). Six representative

PAH recommended by the World Health Organization (1970) as the measure of PAH contamination in drinking water was monitored in this study (BbFL was replaced by BjFL) and the average concentration of PAH was found to be 13.5 ng/l. The U.S. EPA also conducted the National Organic Monitoring Survey (NOMS, 1977) to determine the frequency of occurrence and the levels of PAH in U.S. drinking water supplies. Of the 110 water samples analyzed, none showed any PAH other than fluoranthene. Seventeen out of 110 samples analyzed showed positive fluoranthene values with an average of 20 ng/l concentration. It should be mentioned that the detection limit of PAH in this study was as high as 50 ng/l. The PAH levels in various drinking waters are shown in Table 4.

Finished waters from various treatment sites are transported to the consumers through a variety of pipelines. Borneff (1977) reported a tenfold increase in PAH concentration from beginning to end of a water supply pipe that resulted from the paint used on the water pipes. Leaching of PAH from the coating materials used on the pipes could possibly cause an increase in their concentration in the water reaching consumers. In other instances, PAH could be adsorbed from the water onto the surface of the pipes causing a decrease in their concentration. In the United States, two kinds of pipes are commonly used as distribution lines for transporting treated waters. These are cast/ductile iron, asbestos/cement pipes and a combination of these. The effect of contact with these pipes on the quality of drinking water in terms of PAH concentration was studied by Basu and Saxena (1977-

TABLE 4

PAH Levels in a Few Drinking Waters

	Concentration, ng/l			
Source	BaP	Carcinogenic PAH	Total PAH	Reference
Mixed tap water at Mainz, Germany			7.0	Borneff, 1964
Water at[a]:				
Syracuse, N.Y.	0.3	0.3	1.1	Basu and Saxena, 1978
Buffalo, N.Y.	0.2	0.2	0.9	Basu and Saxena, 1978
New York, N.Y.	0.5	3.9	6.4	Basu and Saxena, 1978
Lake George, N.Y.	0.3	1.5	4.2	Basu and Saxena, 1978
Endicott, N.Y.	0.2	1.1	8.3	Basu and Saxena, 1978
Hammondsport, N.Y.	0.3	1.5	3.5	Basu and Saxena, 1978
Pittsburgh, Pa.	0.4	1.9	2.8	Basu and Saxena, 1978
Philadelphia,Pa.	0.3	2.0	14.9	Basu and Saxena, 1978
Huntington, W. Va.	0.5	2.0	7.1	Basu and Saxena, 1978
Wheeling, W. Va.	2.1	11.3	138.5	Basu and Saxena, 1977-78
New Orleans, La.	1.6	1.6	2.2	Basu and Saxena, 1978
Appleton, Wis.	0.4	2.4	6.1	Basu and Saxena, 1977-78
Champaign, Ill,	N.D.[b]	1.2	2.8	Basu and Saxena, 1977-78
Fairborn, Ohio	0.1	0.8	2.9	Basu and Saxena, 1977-78
Elkhart, Ind.	N.D.[b]	0.3	0.3	Basu and Saxena, 1977-78

[a] Only the six WHO (1970) - recommended PAH were analyzed, with the exception that BbFL replaced BjFl. PAH were concentrated by passing 60 liters of drinking water through polyurethane foams. The eluate from the foams was subjected to cleanup and analyzed for PAH by TLC-spectrofluonmetry.

[b] N.D.: not detected.

78). Because of the intermixing of the pipes, it is difficult to draw definite conclusions from their results. However, it seems likely that in instances where an enhancement of PAH concentration was observed, the tar/asphalt coating of the pipes was responsible for the increase. Cement-coated pipes, on the other hand, produced lower PAH concentrations, possibly due to adsoprtion of PAH from the water.

There are very few epidemiological studies concerning the correlation between cancer and drinking water. It was, nevertheless, noted that four London boroughs, supplied largely by well water, had lower cancer mortalities than most of the other boroughs, which were supplied with surface water (Stocks, 1947). Another study concluded that the highest cancer death rates occurred in communitites supplied by river water, followed by communities supplied by well water, and health water (Diehl and Tromp, 1953; Tromp, 1955). However, none of these studies attempted to correlate cancer morbidity with concentrations of PAH. Finally, it should be noted that one epidemiological study of the incidence of gastric cancer concluded that social factors and the kinds of soils present reduced the correlations otherwise obtained with the type of domestic water supply (Wynne-Griffith and Davies, 1954; Davies and Wynne-Griffith, 1954).

Although the levels of PAH detected in U.S. drinking waters are well below the WHO (1970) recommended limit of 200 parts per trillion (ppt), the health hazards associated with repeated exposure (more effective than an equivalent single dose (Payne and Hueper, 1960) of carcinogens through drinking water should not be underestimated. Shabad and Il'nitskii (1970) stated that the amount of carcinogenic PAH consumed by man from water is typically only 0.1 percent of the amount he consumes from foods. If the total PAH uptake from food is taken as 4.15 mg/year (Borneff, 1977), the human uptake of PAH from drinking water should not exceed 4 µg/year. Assuming the PAH concentration value of 13.5 ng/l in U.S. drinking water (Basu and Saxena, 1978; Basu and Saxena, 1977-78), and a daily consumption of 2.5 liters of drinking water, the yearly intake of PAH from U.S. drinking would be 12.3 µg/ or 0.3 percent of the total food intake. Nevertheless, the accumulation of PAH in edible aquatic organisms through polluted surface waters can greatly increase their amount in foods, including fish, some mollusks, and edible algae (Andelman and Snodgrass, 1974). The use of contaminated water for irrigation can also spread PAH into other vegetable foodstuffs (Shabad and Il'nitskii, 1970). Therefore, it is important to monitor the PAH levels in surface waters not used as drinking water sources as well as drinking waters, in order to estimate accurately the human intake of PAH.

Ingestion from Foods

PAH formed through both natural and man made sources can enter the food chain of man. There is considerable disagreement, however, concerning the contribution of each of these sources to the total PAH contamination in foods.

From their work with marine algae and fishes obtained from polluted and unpolluted sources, Harrison, et al. (1975) concluded that endogenous synthesis may be the important factor for PAH contamination in these species. Others, however, believe that the endogenous formation of PAH occurs to such a limited extent that it is completely masked by the accumulation of PAH from the environment (Payer, et al. 1975). The latter conclusion was verified by Shabad and Smirnov (1972). It has been demonstrated by these authors that plants near an airport contained to 10 to 20 times more BaP than areas remote from the runway. The results of Dunn and Stich (1976) indicated a correlation between the PAH level in mussels with industrial, urban, and recreational activity. The highest occurrence of BaP in marine organisms in the areas adjacent to the sea lanes tends to support the view that exogenous sources are the predominant factor for PAH contamination in foods.

The primary routes of entry for PAH in foods are surface adsorption and biological accumulation from the environment (Binet and Malet, 1963). The adsorption of PAH from the soil by various plant roots and translocation to the shoots is well documented (Lo and Sandi, 1978). Similarly, the absorption of PAH by other marine organisms has been demonstrated by Lee, et al. (1972). Oysters and clams collected from moderately polluted waters also concentrate PAH via absorption (Cahnmann and Kuratsune, 1957; Guerrero, et al. 1976). The waxy surface of some plant leaves and fruits can concentrate PAH through surface adsorption (Hetteche, 1971 and Kolar, et al. 1975). Kolar, et al. (1975) have shown that the concentration of BaP in vegetation is proportional to the exposure time during the growing season (bioaccumulation through adsorption) and the structure of the surface of the plant (surface adsorption). The above-ground parts of the vegetables contain move BaP than underground parts. Only about ten percent of the externally deposited BaP in lettuce, kale, spinach, leeks, and tomatoes can be removed by cold water washing (Kolar, et al. 1975).

Food additives and food packaging materials such as paraffin waxes containing PAH, contribute to the enhancement of PAH levels in processed foods. For example, Swallow (1976) found that paraffin wax wrapping for food contained PaA, CH, BeP, and BaP at levels of 29 ppb, 2 ppb, 0-48 ppb, and 2 ppb, respectively. Certainly, some of these PAH in the packing material can diffuse into the food. Hexane, a commercial solvent used to extract edible vegetable oils, is also a source of PAH contamination. PAH present in food-grade carbon blacks used for food processing can be transported to the food products. Curing smoke and other pyrolysis products used during cooking add to the level of PAH in food. However, in raw foods which require cooking, the largest source of PAH contamination originates from the cooking process itself.

In order to summarize the available data on PAH levels, various foods have been categorized following the pattern of USDA-FDA for total diet samples (Martin and Duggan, 1968). These are shown in Tables 5 through 11. It should be recognized that the data presented in the tables are neither exhaustive nor absolute. Not all the PAH detected by the various authors are listed in these tables. Only the most frequently detected PAH are listed. The concentration values given in these tables are subject to considerable variation. The PAH concentrations in uncooked foods largely depend on the source of food. For example, vegetables, fruits, and fishes obtained from a polluted environment can be expected to contain higher concentrations of PAH. Therefore, the PAH content is subject to regional variation. In the case of raw foods which require cooking, the method of cooking is largely responsible for the PAH content in the food and is subject to regional or even personal variation. Therefore, the frequency of occurrence of PAH in a particular food is dependent on a number of factors. The results presented in Tables 5 and 6 represent only the values where the sample showed detectable levels of PAH.

It has been claimed by Zitko (1975) that PAH are not bioaccumulated along the food chain. However, Bjørseth (1978) demonstrated that both common and horse mussels bioaccumulated PAH, although not to the same degree. Dunn and Stitch (1976) have shown that mussles cannot metabolize BaP upon their removal from water. In water, mussels released 79 percent of naphthalene in 3 days, with a half-life of 1.3 days. The BaP released from both clams and mussels in water takes place with a half-life of two to five weeks (Dunn and Stitch, 1976).

The human intake of PAH through the digestive system has been estimated by Borneff (1977). According to this

TABLE 5

PAH Concentrations (ppb) in a few Vegetable Oils and Margarine

	A	PA	FL	P	BaA	BeP	BaP	PR	BPR	CH
Corn[a]				3.1	0.8	0.7	0.7		0.6	
Coconut[b]	36	51	18	15		2	2			12
Margarine[c]					1.4-29.5	0.5-1.2	0.2-6.8			
Sunflower[c]					13[d]	4	8		4	
Soybean[a]			1.3	1.6	0.9	1.6	1.4		1.0	
Olive[a]			3.2	2.6	1.0	0.4	0.5		0.9	
Peanut[a]			3.3	2.9	1.1		0.6		0.9	

[a] Howard, et al. 1966c

[b] Beirnoth and Rost, 1967

[c] Swallow, 1976

[d] This value represents concentration of BaA and CH

TABLE 6

PAH Concentrations (ppb) in Smoked and Non-Smoked Fish

Fish	F	A	PA	FL	P	BaA	BeP	BaP	PR	BPR
Smoked[a] Eel	9	4	37	4	6		t[b]	1.0		
Smoked[a] Lumpfish	5	t	10	2	1	t	t	0		
Smoked[a] trout	67	26	52	12	5		t	t		
Smoked[b] herring				3	2.2					
Smoked[b] herring (dried)				1.8	1.8	1.7	1.2	1.0		1.0
Smoked[b] salmon				3.2	2.0	0.5	0.4			
Smoked[b] sturgeon				2.4	4.4			0.8		
Smoked[b] whitefish				4.6	4.0			4.3		
Smoked[c] whiting					<0.5			6.6	0.7	2.4
Smoked[d] redfish		1.5	4.1	4.0	3.0		0.3	0.3		
Smoked[d] cod					0.6			4.0	0.4	2.2
Electric smoked mackerel[d]	2.6	1.9	9	5.2	3.6	1.2	0.5	0.2	t	0.2
Gas smoked mackerel[d]	8.2	2.3	11	2.6	4.0	0.6	0.2	0.3	t	0.3
Non-smoked haddock[b]				1.6	0.8					
Non-smoked[b] herring (salted)				0.8	1.0					
Non-smoked salmon				1.8	1.4					

[a] Thorsteinsson, 1969; Dungal, 1961

[b] Howard, et al. 1966a

[c] Malanoski, et al. 1968

[d] Masuda and Kratsune, 1971

estimate the human intake of PAH per year is about 3 to 4 mg from fruits, vegetables, and bread, 0.1 mg from vegetable fats and oils, and about 0.05 mg from smoked meat or fish and drinking water.

Vegetable Fats, Oils, and Shortening: Several PAH have been found in edible oils by European workers (Howard and Fazio, 1969). The PAH levels in a few vegetable oils and margarine are presented in Table 5. PAH other than those shown in Table 5 have been reported in these oils (Swallow, 1976). Since the concentration of PAH in vegetable oils depends on the nature of refinement of the crude oil (Grimmer and Hildebrandt, 1967), one can expect variations in their concentrations. Heating of the oils also leads to a slight increase in PAH concentrations. For example, Lijinsky and Shubik (1965b) did not detect any PAH in uncooked Wesson and Crisco oil. However, oil used previously for deep-frying of food showed 1.4 ppb BaP, 12 ppb FL, and 6 ppb pyrenes (Lijinsky and Ross, 1967; Malanoski, et al. 1968).

Swallow (1976) determined the level of PAH in butter and found the concentration of BaA + CH, BaP, IP + DBA, and BPR to be 1 ppb. In a total diet study with a composite sample containing the fats, oils, and shortening, Howard, et al. (1968b) found less than 0.5 ppb of seven PAH. However, Borneff (1977) estimated that the human intake of PAH from vegetable fats and oils amounted to 0.1 mg per year.

Fish and Other Marine Foods: Raw fish from unpolluted waters usually do not contain detectable amounts of PAH, but smoked or cooked fish contain varying levels of PAH. In addition to the origin of the fish, (polluted or unpolluted water), the amount of PAH in smoked fish depends on various parameters, such as type of smoke, temperature of combustion, and degree of smoking (Draudt, 1963).

The skin of fish apparently serves as a barrier to the migration of PAH into the body tissues. This was postulated by Malanoski, et al. (1968) from their observations that the BaP level in the skin was much higher than in the interior of cooked fish.

The PAH levels in various smoked and unsmoked fish are shown in Table 6. In addition to the fishes presented in this table, various other marine organisms had been tested for PAH content. For example, cooked squid and prawns had BaP concentrations of 1.04 ppb and 0.08 ppb, repsectively (Shiraishi, et al. 1975). Various other edible marine organisms were investigated and found to contain PAH. Swallow (1976) analyzed smoked oysters and determined the levels of BaA + Ch, BbFl + BkFL + BjFL, IP + DBA and BPR to be 19 ppb, 8 ppb, 9 ppb, 7 ppb and 3 ppb, respectively. Cooked scallops were found to contain 9.9 ppb BaP (Shiraishi, et al. 1975). Shiraishi, et al. (1973) detected 0 to 31.3 ppb BaP in various Japanese seaweeds. However, no BaP was detected in crab (Shiraishi, et al. 1975). The absence of BaP in crab is corroborated by the work of Lee, et al. (1976), who found no evidence of PAH storage by any of the crab tissues.

A bioconcentration factor (BCF) relates the concentration of a chemical in water to the concentration in aquatic organisms, but BCF's are not available for the edible portions of all four major groups of aquatic organisms consumed in the United States. Since data indicate that the BCF for lipid-soluble compounds is proportional to percent lipids, BCF's can be adjusted to edible portions using data on percent lipids and the amounts of various species consumed by Americans. A recent survey on fish and shellfish consumption in the United States (Cordle, et al. 1978) found that the per capita consumption is 18.7 g/day. From the data on the nineteen major species identified in the survey and data on the fat content of the edible portion of these species (Sidwell, et al. 1974), the relative consumption of the four major groups and the weighted average percent lipids for each group can be calculated:

Group	Consumption (Percent)	Weighted Average Percent Lipids
Freshwater fishes	12	4.8
Saltwater fishes	61	2.3
Saltwater molluscs	9	1.2
Saltwater decapods	18	1.2

Using the percentages for consumption and lipids for each of these groups, the weighted average percent lipids is 2.3 for consumed fish and shellfish.

No measured steady-state bioconcentration factor (BCF) is available for any of the following compounds except BaP (Lu, et al. 1977), but the equation "Log BCF = 0.76 Log P - 0.23" is commonly used (Veith, et al. Manuscript) to estimate the BCF for aquatic organisms that contain about eight percent lipids from the octanol-water partition coefficient (P). An adjustment factor of 2.3/8.0) = 0.2875 can be used to adjust the estimated BCF from the 8.0 percent lipids on which the equation is based to the 2.3 percent lipids that is the weighted average for consumed fish and shellfish. Thus, the weighted average bioconcentration factor for the edible portion of all aquatic organisms consumed by Americans can be calculated (Table 6a):

TABLE 6a

Calculated Bioconcentration Factors of PAH
Based Upon the Octanol-water Partition Coefficient

Compound	P	BCF	Weighted BCF
Acenaphthylene	5,500	410	120
Anthracene	28,000	1,400	410
Benz(a)anthracene	410,000	11,000	3,100
Benzo(b)fluoranthene	1,100,000	24,000	6,800
Benzo(k)fluoranthene	1,100,000	24,000	6,800
1,12-benzoperylene	3,200,000	52,000	15,000
Benzo(a) pyrene	1,150,000	24,000	6,800
Chrysene	410,000	11,000	3,100
Dibenz(a,h)acridine	540,000	13,000	3,800
Dibenz(a,h)anthracene	5,900,000	82,000	24,000
Dibenzofuran	13,000	800	230
Fluoranthene	79,000	3,100	900
Fluorene	15,000	880	250
1-methylphenanthrene	100,000	3,700	1,100
Phenanthrene	28,000	1,400	410
2,3-phenylene pyrene	3,200,000	52,000	15,000
Pyrene	76,000	3,000	870

Caution must be excercised in application of common practice in obtaining BCF described above, because the ecological impact of PAH is not well understood at this time. Numerous studies show that despite their high lipid solubility, PAH show little tendency for bioaccumulation in the fatty tissues of animals or man (Lee, et al. 1972; Ahokas, et al. 1975). This observation is not unexpected, in light of convincing evidence to show that PAH are rapidly and extensively metabolized. Since only low levels of PAH are detected in plants and lower organisms, (Radding, et al. 1976), transfer of PAH through the food chain does not seem likely. The direct impact of PAH on plants, animals, or the ecological balance of nature is difficult to evaluate, since few data are available which suggest that adverse effects may occur.

Meat and Meat Products: Raw meat does not normally contain PAH, but smoked or cooked meat may contain varying amounts of PAH (Lo and Sandi, 1978). Table 7 shows the concentration of PAH detected in a few meats and meat products. The higher concentration of PAH in charcoal broiled ribs (containing more fats) than in charcoal broiled steaks tends to support the idea that the most likely source of PAH is the melted fat. These fats drip on the heat source and are pyrolyzed. The PAH compounds in the smoke are then deposited on the meat as the smoke rises (Lijinsky and Shubik, 1965a). Many factors, such as degree of smoking, and the temperature of combustion affect the composition and concentration of PAH in cooked meat (Howard, et al. 1966a) In addition to the pyrolysis of fats, incomplete combustion of charcoal

TABLE 7

PAH Concentrations (ppb) in a Few Smoked Meat and Meat Products

Meat	A	PA	FL	P	BaA	BeP	BaP	PR	BPR	CH
Charcoal broiled steaks[a]		21.0	43.0	35.0	1.4	5.5	5.8	0.9	6.7	0.6
Barbecued ribs[a]	7.1	58.0	49.0	42.0	3.6	7.5	10.5	1.5	4.7	2.2
Smoked beef (chipped)[b]			0.6	0.5	0.4					
Smoked ham[b]			14.0	11.2	2.8	1.2	3.2		1.4	
Smoked pork (roll)[b]			3.1	2.5						
Smoked frankfurters[b]			6.4	3.8	1.5		2.0			
Barbecued beef[e]			2.0	3.2	13.2	1.7	3.5		4.3	9.6
Smoked hot sausages[c]				1.5	0.5		0.4			1.0
Smoked mutton[d]	13.0	104.0	18.0	8.0	2.0	5.0	t[h]			
Smoked mutton sausages[d]	2.0	17.0	6.0	2.0	0.5	t	t			
Smoked bologna[e]					0.04-0.55	5.0	0.04-0.08	0.04-0.07	0.04-0.20	0.15-1.20[i]
Smoked salami[f]	0.7	D[g]	5.6	5.2	0.6	0.2	2.0 0.8	3.2	D	1.2
Smoked Mortadella[f]	2.6	D	22.0	15.0	2.8	1.8	0.7	0.1	0.4	3.4
Heavily smoked bacon[f]	20.0	D	35.0	27.0	29.0	D	3.6	0.9	3.0	D

[a] Lijinsky and Shubik, 1965a

[b] Howard, et al. 1966a,b; Panalaks, 1976

[c] Malanoski, et al. 1968

[d] Thorsteinsson, 1969

[e] Frethein, 1976; Panalaks, T., 1976

[f] Lo and Sandi, 1978

[g] D: detected

[h] t: trace

[i] compound unseparated

can also contribute to the PAH content in broiled meat. Thus, the source of heat used for cooking is responsible for the PAH concentration in cooked meats. These effects are indicated in Table 8.

In North America, except for smoked ham, most smoked meats contained much less carcinogenic PAH than European samples (Howard, et al. 1966a,b). The high incidence of stomach carcinoma in Iceland has been explained by the high concentration of BaP in smoked trout and mutton which are consumed in large quantities in the area (Bailey and Dungal, 1958). On the other hand, very low concentrations of PAH in Norwegian bologna sausages (see Table 8) are probably indicative of the tradition of light smoking of food in Norway (Fretheim, 1976).

About 60 to 75 percent of the BaP in smoked food has been found to be in the superficial layer of meat (Thorsteinsson, 1969). This low penetration has also been noted by Rhee and Bratzler (1970), who observed that in smoked bologna sausages, the BaP is located within 1.5 mm from the surface. Cellulose casings can be used as a more effective barrier to BaP permeation during smoking of frankfurters than animal casing (Simon, et al. 1969).

In addition to meat and meat products, liquid smoke flavorings used during the cooking of meat have been found to contain a variety of PAH. Lijinsky and Shubik (1965b) have detected BaP, FL, P, BPR, BaA, and CH in liquid smoke at concentrations of 1 ppb, 16 ppb, 7 ppb, 1 ppb, 12 ppb, and 6 ppb, respectively. In hickory liquid smoke flavoring,

TABLE 8

Effect of Different Cooking Variables on the Concentration of PAH (ppb) in Cooked Meat

Meat	Effect	FL	P	BaA	BeP	BaP	BPR	CH	CR
Charcoal broiled hamburger[a]	Fat Content								
Fat[c], hot[d]		13.3	7.7	2.7		2.6	14.9	1.7	1.0
Lean[e], hot		0.3	1.6				0.9		
No-drip pan		0.2	0.1				t	t	
Charcoal broiled hamburger[a]	Heating temperature								
Lean[f], hot		0.3	1.6				0.9	0.3	
Lean, cool		1.3	0.6						
Broiled T-bone steak[a]	Heat source								
Charcoal, hot		19.8	19.1	31.0	17.6	50.4	12.4	25.4	8.0
Flame, hot		19.0	20.0	3.9	5.7	4.4	6.2	2.0	9.0
Smoked ham[b]	Degree of Smoke								
Light		4.0-14.0	2.0-11.0	0.5-3.0	0-2.0	3.0-4.0	0-1.4	0-3.0	
Heavy		48.0-156.0	35.0-161.0	6.0-33.0	4.0-26.0	3.8-55.0	2.5-25.0	12.0-66.0	

[a] Lijinsky & Ross, 1967
[b] Filipovic & Toth, 1971; Toth & Blass, 1972
[c] Fat: 21% fat
[d] Hot: 7 cm. from heat source
[e] Lean: 7% fat
[f] cool: 25 cm from heat source

Youngblood, and Blumer (1975) found the total concentration of PAH as 9,400 ppm. The high level of PAH present in the resinous condensate in liquid smoke flavoring indicates the importance of its efficient removal from the aqueous flavoring prior to its use in foodstuffs (White, et al. 1971).

Vegetables, Fruits, Grains and Cereal Products, Sugar and Adjuncts, and Beverages: Various European and Japanese workers have reported the presence of BaP and other PAH in these products; their results are summarized in Tables 9 to 11. Studies in this field in North America are lacking. Test results indicate that surface adsorption and root uptake are the principal modes of PAH accumulation in vegetables (Binet and Mallet, 1963). The frizzy leaf of kale, for example, has a large surface area and holds dust particularly well. PAH are adsorbed by the wax layer and protected against solar reactions (Hetteche, 1971). In kale, Hetteche (1971) found the concentration of PAH to be the following: PA, 70-586 ppb; A, 2.4-97.5 ppb; P, 36.2-510 ppb; FL, 53.6-1196 ppb; BaA, 11.2-230 ppb; CH, 28.6-395 ppb; BeP, 3.8-67.2 ppb; BaP, 0.9-48.6 ppb; PR, N.D.-7 ppb; BPR, 1.2-46.4 ppb; and CR 0.1-7.2 ppb.

The concentration of BaP in vegetables is directly proportional to exposure time during the growing season and structure of the surface of the plant. The above-ground parts contain more BaP than underground parts. Washings with cold water do not remove more than ten percent of the BaP (Kolar, et al. 1975). Fruits grown in polluted environ-

TABLE 9

BaP content in Fruits, and Other Foods

Fruits	Concentration (ppb)	Comments	References
Apple	0.02		Shiraishi, et al. 1975
Apple	8.3	Polluted environment	Kolar, et al. 1975
Banana	0.02		Shiraishi, et al. 1975
Banana peel	0.03		Shiraishi, et al. 1975
Grape	0.2	Polluted environment	Kolar, et al. 1975
Grape	0.02		Shiraishi, et al. 1975
Japanese pear	0.05		Shiraishi, et al. 1975
Pear	1.9	Polluted environment	Kolar, et al. 1975
Persimmon	0.02		Shiraishi, et al. 1975
Pineapple	0.02		Shiraishi, et al. 1975
Plums	0.04		Shiraishi, et al. 1975
Plums	29.7	Polluted environment	Kolar, et al. 1975
Dried Prunes	0.2 to 1.5		IARC, 1973
Manderin Orange	0.03		Shiraishi, et al. 1975
Orange peel	0.15		Shiraishi, et al. 1975
Strawberry	N.D.[a]		Shiraishi, et al. 1975
Pumpkin	N.D. to trace		Shiraishi, et al. 1974

Grains & Cereal Products

Product	Concentration (ppb)	Comments	Reference
Wheat grain	0.1	Polluted environment	Kolar, et al. 1975
Wheat sprouts	60.0		Siddiqui and Wagner, 1972
Cereals	0.2 to 4.1		IARC, 1973
Barley	0.3	Polluted environment	Kolar, et al. 1975
Oats	0.2	Polluted environment	Kolar, et al. 1975
Polished rice	N.D.[a]		Shiraishi, et al. 1973
Rye seedling	10.0 to 20.0	8 other PAH identified	Graf and Nowak, 1966
Lentil seedlings	10.0 to 20.0	8 other PAH identified	Graf and Nowak, 1966
Sesame seeds	N.D.		Shiraishi, et al. 1973

Sugar and Adjuncts

Product	Concentration (ppb)	Comments	Reference
Charred biscuits	11.0-72.0		Kuratsune, 1956
Caramel	N.D.[a]		Shiraishi, 1973
Chocolate	0.2-1.7	4 other PAH quantified	Fabian, 1969

[a] N.D.: not detected

TABLE 10

Concentration (ppb) of a BaP in a Few Vegetables

Vegetable	Concentration	Comments	References
Parsley leaf and stem	24.3	Polluted environment	Kolar, et al. 1975
Red clover	7.5	Polluted environment	Kolar, et al. 1975
Mushroom	7.0	Polluted environment	Kolar, et al. 1975
Lettuce	8.6	Polluted environment	Kolar, et al. 1975
Lettuce	N.D.		Shiraishi, et al. 1974
Spinach	6.2	Polluted environment	Kolar, et al. 1975
Spinach	1.3		Shiraishi, et al. 1973
Spinach	7.4		IARC, 1973
Radish leaves	5.3	Polluted environment	Kolar, et al. 1975
Radish roots	1.2	Polluted environment	Kolar, et al. 1975
Radish roots	N.D.[a]		Shiraishi, et al. 1974
Tomatoes	0.1	Polluted environment	Kolar, et al. 1975
Tomatoes	0.2		IARC, 1973
Cabbage	12.3 to 20.9	Polluted environment	Kolar, et al. 1975
Cabbage	N.D.		Shiraishi, et al. 1974
Chinese cabbage	0.05		Shiraishi, et al. 1974
Potatoes	N.D. to 0.01		Shiraishi, et al. 1974
Potatoes	0.2	Polluted environment	Kolar, et al. 1975
Sweet potatoes	N.D.		Shiraishi, et al. 1974
Sweet pepper	N.D.		Shiraishi, et al. 1974
Cauliflower	5.1	Polluted environment	Kolar, et al. 1975
Bean paste	N.D.		Shiraishi, et al. 1973
Kidney bean	N.D.		Shiraishi, et al. 1973
Carrot	N.D. to 0.02		Shiraishi, et al. 1973
Cucumber	N.D.		Shiraishi, et al. 1973
Eggplant	N.D.		Shiraishi, et al. 1973
Onion bulb	N.D. to 0.01		Shiraishi, et al. 1974
Onion greens	0.01		Shiraishi, et al. 1974

[a] N.D.: not detected

TABLE 11

BaP Concentrations (ppb) in Beverages

Beverage	Concentration	Comments	References
Dark rum	1.0		Swallow, 1976
Whiskey	0.04	3 quinolines detected	IARC, 1973; Nishimura and Masuda, 1971
Tea leaves	3.9 to 21.3		IARC, 1973
Black tea aroma[a]		7 quinolines detected	Vitzthum, et al. 1975
Roasted coffee (moderate dark)	N.D.		Kuratsune and Hueper, 1960
Roasted coffee (darkest)	N.D. to 4.0		Kuratsune and Hueper, 1958, 1960
Coffee soots[b]	200.0-440.0		Kuratsune and Hueper, 1958

[a] This is the volatile components of black tea.

[b] These are the soots generated during direct and indirect roasting of coffee beans.

ments show a high degree of PAH contamination mainly through adsorption on the waxy surface.

In smoked Gouda cheese, Panalaks (1976) found 0.5 ppb BaP and Howard, et al. (1966a) found 2,8 ppb FL and 2.6 ppb P. The unsmoked cheese contained lower levels of PAH (1966a). Grimmer (1974) analyzed baker's yeasts and determined the level of PAH. The values are shown in Table 12.

Inhalation

A variety of PAH have been detected in ambient air in the United States and elsewhere in the world. Because of its carcinogenic properties, BaP has been most extensively monitored and has frequently been used as an indicator of ambient PAH. The presumed correlation between the concentration of BaP and other PAH, however, does not always exist. For example, a study by Kertesz-Saringer and Morlin (1975) found little or no relationship between BaP and other PAH in Budapest air. Gordon (1976) and Gordon and Bryan (1973) came to a similar conclusion from their work with ambient Los Angeles air.

The concentration and the nature of PAH in ambient air are dependent on a number of factors. In general, the PAH concentration is lowest during the summer months and highest during the winter, (Sawicki, et al. 1962) probably due to commercial and residential heating during winter (U.S. EPA, 1974). However, there are some exceptions. Cleveland, for instance, does not follow the high winter-low summer pattern (U.S. EPA, 1974). It has been suggested that this may be due to significant industrial emissions that are uniform throughout the year (U.S. EPA, 1974).

TABLE 12

PAH Concentrations (ppb) in a Variety of Baker's Yeast[a,b]

PAH	French	German	Scottish	Russian
PA	17.8-34.60	67.0	1620	7.2
A	2.6-13.6	4.8-10.2	567	4.7
P	11.6-19.6	11.5-35.0	327	16.9
FL	18.5-21.2	17.2-66.8	93	32.1
BaA	9.8-23.3	2.5-15.8	203	10.8
CH	8.1-13.4	4.2-14.0	50	11.1
BeP	8.0-10.6	3.1-14.3	40.4	8.7
BaP	8.0-12.2	1.8-13.2	6.2	0.5
PR	0.9-1.2	N.D.-0.5	16.7	6.0

[a] Grimmer, 1974

[b] This is baker's yeast as opposed to dietary or brewer's yeast.

The nature and relative amounts of individual PAH in ambient air are also dependent on the source of these compounds. Thus, the content of PAH sampled in an industrial area is a composite of the emissions from various industrial and transportation sources within the area. For example, Gordon (1976), from his study of the relative PAH concentration pattern for different areas in Los Angeles, found a correlation between coronene concentration and automobile emissions. Similarly, Greinke and Lewis (1975) had demonstrated that emissions from coke ovens contain lower amounts of certain methyl-substituted PAH than emissions from petroleum pitch volatiles. Bartle, et al. (1974) also used a PAH profiling technique for the identification of air pollution sources, such as coal burning, vehicular emissions, and oil and gas burning.

Meteorological factors have a dominant effect on PAH concentrations. For example, Lunde and Bjørseth (1977) demonstrated that under favorable wind conditions PAH from downtown London could be transported to Norway. The tendency of atmospheric inversion to increase the PAH levels in urban areas has also been shown (Hoffmann and Wynder, 1977).

The annual average ambient BaP concentrations for different U.S. urban and rural locations during the period 1966-70 have been compiled by U.S. EPA report (Santodonato, et al. 1978). The average BaP concentrations in U.S. urban and rural areas obtained from this U.S. EPA study are shown in Table 13.

TABLE 13

Average BaP Concentrations (ng/m^3) in U.S. Urban and Rural Areas During 1966-76[a]

Period	1966	1970	1976
Urban	3.2	2.1	0.5
Rural	0.4	0.2	0.1[b]

[a] Santodonato, et al. 1978
[b] This value is the average of two rural locations.

An interesting trend has developed from the National Air Surveillance Network (NASN) monitored BaP values listed in Table 13. As can be seen, the average BaP concentrations in urban areas decreased from 3.2 ng/m^3 in 1966 to 2.1 ng/m^3 in 1970, approximately a 30 percent decrease. The decrease is more dramatic (i.e., >80 percent) between the period 1966 to 1976. Even the concentrations in rural areas indicate a downward trend. This decline in BaP concentration is believed to be due primarily to decreases in coal consumption for commercial and residential heating, improved disposal of solid wastes, and restrictions on open burning (Faoro and Manning, 1978). A further observation that can be made from Table 13 is the five- to tenfold difference in BaP concentration between urban and rural locations.

The NASN study did not include the determination of concentrations of other PAH. The summer and winter averages of ambient PAH concentrations for seven urban locations were determined by Sawicki, et al. (1962). The averages of summer and winter data from this work are presented in Table 14.

TABLE 14

Summer-Winter Average of Ambient PAH Concentrations (ng/m^3) in the Air of Selected Cities[a]

City	BPR	BaP	BeP	BkFL	P	CR	PR	A	Total
Atlanta	7.0	4.5	3.1	3.7	3.4	3.4	0.8	0.4	26.3
Birmingham	13.2	15.7	8.0	8.8	9.6	3.0	3.8	1.3	63.4
Detroit	21.3	18.5	14.2	12.5	19.4	4.1	3.9	1.2	95.1
Los Angeles	10.2	2.9	4.4	3.1	3.2	7.1	0.8	0.1	31.8
Nashville	10.2	13.2	7.6	8.0	15.3	3.0	2.3	1.0	60.6
New Orleans	6.0	3.1	4.8	2.9	1.3	14.8	0.6	0.1	33.6
San Francisco	5.1	1.3	1.7	1.0	1.0	3.3	0.2	0.1	13.7

[a] Sawicki, et al. 1962

The average of total PAH concentrations for all cities listed in Table 14 is 46.4 ng/m^3. However, these values were obtained from ambient air sampled in 1958-59 and probably have decreased during subsequent years. If an 80 percent decrease of total PAH concentration is assumed (as in the case of BaP), the present ambient PAH concentration in the U.S. urban areas can be extrapolated as 9.3 ng/m^3. Although the concentration of BaP and some other PAH might have decreased in past decades, the concentration of corenene and some other PAH may not have maintained the same trend. This could be due to the higher number of automobiles in current use. Therefore, this 80 percent decrease figure may or may not be valid for all PAH.

The concentrations of PAH in recent years in individual U.S. cities have been determined by a number of authors. The lowest and highest values of these determinations published during the period 1971-77 are shown in Table 15.

TABLE 15

PAH Concentration Range in U.S. Cities Determined by Various Authors in Recent Years

Compound	Concentration, range, ng/m^3	Reference
NA	0.052 - 0.350	Krstulovic, et al. 1977
A	0.068 - 0.278[a]	Lunde and Bjørseth, 1977
BaA	0.18 - 4.6	Fox and Staley, 1976; Gordon, 1976
PA	0.011 - 0.340	Krstulovic, et al. 1977
FL	0.10 - 4.1	Fox and Staley, 1976; Hoffman and Wynder, 1977
BbFL	0.1 - 1.6	Gordon and Bryan, 1973
BjFL	0.01 - 0.8	Gordon and Bryan, 1973
BkFL	0.03 - 1.3	Gordon and Bryan, 1973
P	0.18 - 5.2	Fox and Staley, 1976; Gordon and Bryan, 1973
BaP	0.13 - 3.2	Colucci and Begeman, 1971; Fox and Staley, 1976
BeP	0.9 - 4.6	Gordon, 1976; Fox and Staley, 1976
IP	0.03 - 1.34	Gordon, 1976; Gordon and Bryan, 1973
CH	0.6 - 4.8	Gordon, 1976; Fox and Staley, 1976
PR	0.01 - 1.2	Gordon and Bryan, 1973
BPR	0.2 - 912	Gordon and Bryan, 1973
CR	0.2 - 6.4	Gordon and Bryan, 1973

[a] This Norwegian value is included because no recent U.S. data are available.

The exact amount of human PAH intake from all modes is difficult to determine because of the different modes of inhalation due to smoking, occupational exposure, or exposure to ambient air. Considering only exposure to ambient air, one needs an average PAH concentration in air in order to determine the PAH intake through inhalation. In the absence of national average data for PAH equivalent to NASN data on national average BaP levels, the yearly average data for Los Angeles are used for the derivation of PAH intake due to inhalation. These values are given in Table 16.

TABLE 16

Average Ambient PAH Concentration in U.S. and Daily Intake of PAH Through Inhalation[a]

PAH	BaP	Carcinogenic PAH[b]	Total PAH
Ambient Conc., ng/m^3	0.5	2.7	10.9
Inahaltion intake/day, ng[c]	5.0	27.0	109.0

[a] These values are based on the study of Gordon, 1976.

[b] Carcinogenicity of PAH are derived from Natl. Acad. Sci. 1972

[c] These values are based on 10 m^3 inhalation of air/day.

It can be seen from Table 16 that the yearly intake of total PAH, carcinogenic PAH, and BaP through inhalation is 39.8 ug, 9.9 ug, and 1.9 ug, respectively. It should be recognized that these data are based on the average ambient air concentration of one city and probably will not reflect the true U.S. average. It is noteworthy, however, that the total ambient PAH concentration of 10.9 ng/m^3 derived from this work is very close to the earlier extrapolated value of 9.3 ng/m^3.

Dermal

No direct information is available on the importance of dermal absorption in total human exposure to PAH. PAH can be absorbed across the skin by animals. For those humans exposed to only ambient levels of PAH, dermal absorption is not likely to be a significant route of entry.

PHARMACOKINETICS

There are no data available concerning the pharmacokinetics of PAH in humans. Nevertheless, it is possible to make limited assumptions based on the results of animal studies conducted with several PAH, particularly BaP. The metabolism of PAH in human and animal tissues has been especially well-studied, and has contributed significantly to an understanding of the mechanisms of PAH-induced cancer.

Absorption

The demonstrated toxicity of PAH by oral and dermal administration (Smyth, et al. 1962) indicates that they are capable of passage across epithelial membranes. The high lipid solubility of compounds in this class supports this observation. Animal studies with structurally-related PAH such as benzo(a)pyrene (BaP), chrysene, 7, 12-dimethylbenz-(a)anthracene (DMBA), benz(a)anthracene, and 3-methylcholanthrene (MCA) confirmed that intestinal transport readily occurs, primarily by passive diffusion (Rees, et al. 1971). In addition, there is ample evidence to indicate that benzo(a)-

pyrene, and presumably other PAH, are easily absorbed through the lungs (Kotin, et al. 1969; Vainio, et al. 1976).

Distribution

The tissue distribution and accumulation of PAH have not been studied in humans. It is known, however, that several PAH (e.g., benzo(a)pyrene, 7, 12-dimethylbenz(a)anthracene, 3-methylcholanthrene, phenanthrene) become localized in a wide variety of body tissues following their absorption in experimental rodents (Kotin, et al. 1969; Bock and Dao, 1961; Dao, et al. 1959; Flesher, 1967). Relative to other tissues, PAH localize primarily in body fat and fatty tissues (e.g., breast) (Schlede, et al. 1970a,b; Bock and Dao, 1961).

Disappearance of BaP from the blood and liver of rats following a single intravenous injection was very rapid (Schlede, et al. 1970a). The concentration of BaP in the blood one minute after a 10 µg injection was 193 ± 29 ng; after five minutes concentration of BaP in the blood was 31 ± ng. Similarly, in the liver, the half-time for BaP disappearance was about ten minutes. In both blood and liver, however, the initial rapid elimination phase was followed by a slower disappearance phase, lasting six hours or more. In the same experiment, disapperance of BaP from the brain was slower than from blood or liver, and the concentration of BaP in fat increased during the six-hour observation period. Schlede and coworkers (1970a) concluded that a rapid equilibrium occurs for BaP between blood and liver, and that rapid disapperance from the blood is due to both metabolism and distribution into tissues. This contention is supported by data (Schlede, et al. 1970b) showing that pretreatment with BaP (which induces microsomal enzyme activity) accelerates both the rate of BaP disappearance from all tissues and the excretion of BaP metabolites into the bile. The ability of BaP to stimulate its own metabolism may have important implications for human situations, where lifelong exposure to PAH is known to occur.

With certain PAH, passage into the fetus following intragastric or intravenous administration to pregnant rats has been variable (Shendrikova and Aleksandrov, 1974).

Metabolism

In the past, the relative lack of chemical reactivity for tumorigenic PAH has been puzzling in light of their dramatic biological effects. Early attempts to explain the carcinogenicity of various PAH utilized physico-chemical calculations (Pullman and Pullman, 1955). These early hypotheses were based on the assumption that those regions of the molecule favoring substitution or addition reactions would preferentially react with critical cellular target sites to initiate a carcinogenic transformation. This concept, however, did not prove successful for PAH.

More recently it was learned that PAH are metabolized via enzyme-mediated oxidative mechanisms to form reactive electrophiles (Lehr, et al. 1978). For many of the PAH, certain "bioactivated" metabolites are formed having the capability for covalent interaction with cellular constituents (i.e., RNA, DNA, proteins) and ultimately leading to tumor formation (see Effects section).

The obligatory involvement of metabolic activation for the expression of PAH-induced carcinogenesis has prompted the investigation of PAH metabolism in numerous animal models and human tissues. From these studies has emerged an understanding of the general mechanisms involved in PAH biotransformation. It is now known that PAH are metabolized by the cytochrome P-450-dependent microsomal mixed-function oxidase (MFO) system, often designated aryl hydrocarbon hydroxylase (Conney, 1967; Marquardt, 1976; Sims, 1976; Gelboin, et al. 1972). The activity of this enzyme system is readily inducible by exposure to chemical and is found in most mammalian tissues, although predominantly in the liver (Bast, et al. 1976; Chuang, et al. 1977; Andrews, et al. 1976; Cohn, et al. 1977; Wiebel, et al. 1975; Grundin, et al. 1973; Zampaglione, et al. 1973). The MFO system is involved in the metabolism of endogenous substrates (e.g., steroids) and the detoxification of many xenobiotics. Paradoxically, however, the MFO system also catalyzes the formation of reactive epoxide metabolites from certain PAH, possibly leading to carcinogenesis in experimental mammals (Sims and Grover, 1974; Selkirk, et al. 1971, 1975; Sims, 1976; Thakker, et al. 1977; Levin, et al. 1977a; Lehr, et al. 1978; see Effects section). A second microsomal enzyme, epoxide hydrase, converts epoxide metabolites of PAH to vicinal glycols, a process which may also play a critical role in carcinogenic bioactivation. Figure 1 presents a schematic representation of the various enzymes involved in activation and detoxification pathways for BaP. At present

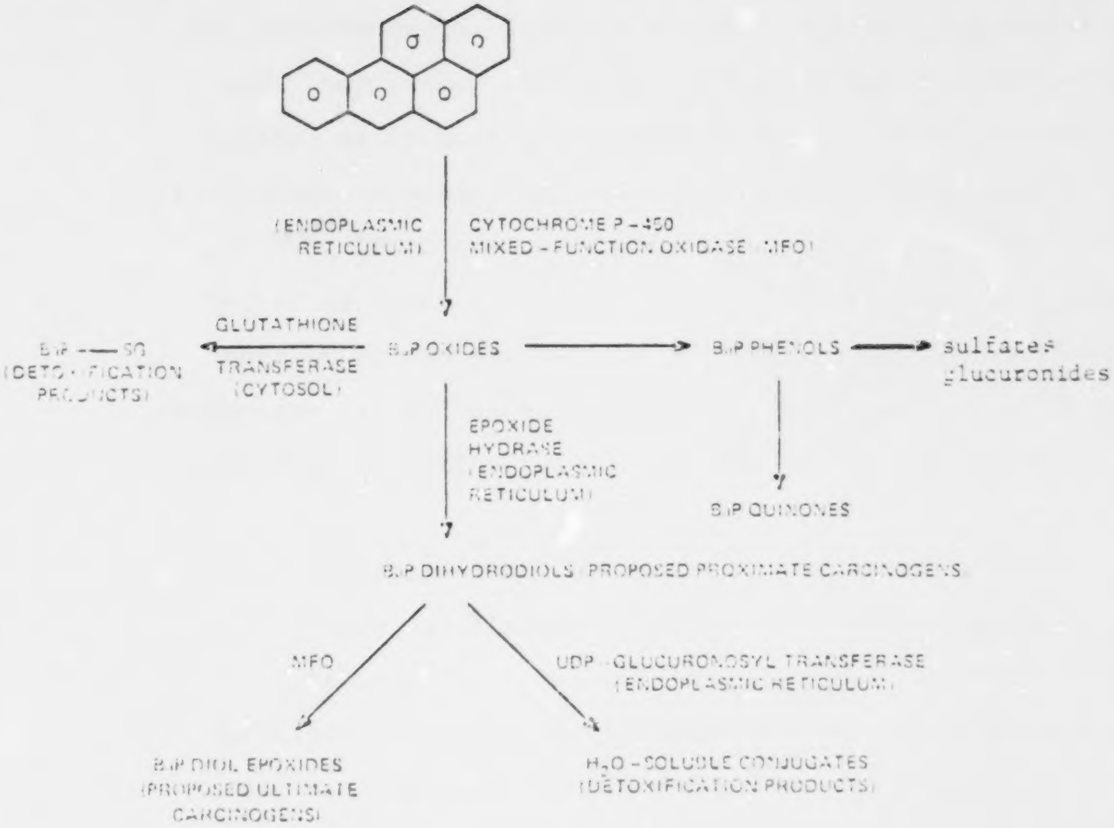
(ENDOPLASMIC RETICULUM)
CYTOCHROME P-450 MIXED-FUNCTION OXIDASE (MFO)
GLUTATHIONE TRANSFERASE (CYTOSOL)
BaP — SG (DETOXIFICATION PRODUCTS)
BaP OXIDES
BaP PHENOLS
sulfates glucuronides
EPOXIDE HYDRASE (ENDOPLASMIC RETICULUM)
BaP QUINONES
BaP DIHYDRODIOLS (PROPOSED PROXIMATE CARCINOGENS)
MFO
UDP-GLUCURONOSYL TRANSFERASE (ENDOPLASMIC RETICULUM)
BaP DIOL EPOXIDES (PROPOSED ULTIMATE CARCINOGENS)
H2O-SOLUBLE CONJUGATES (DETOXIFICATION PRODUCTS)

Figure 1. Enzymatic pathways involved in the activation and detoxification of BaP.

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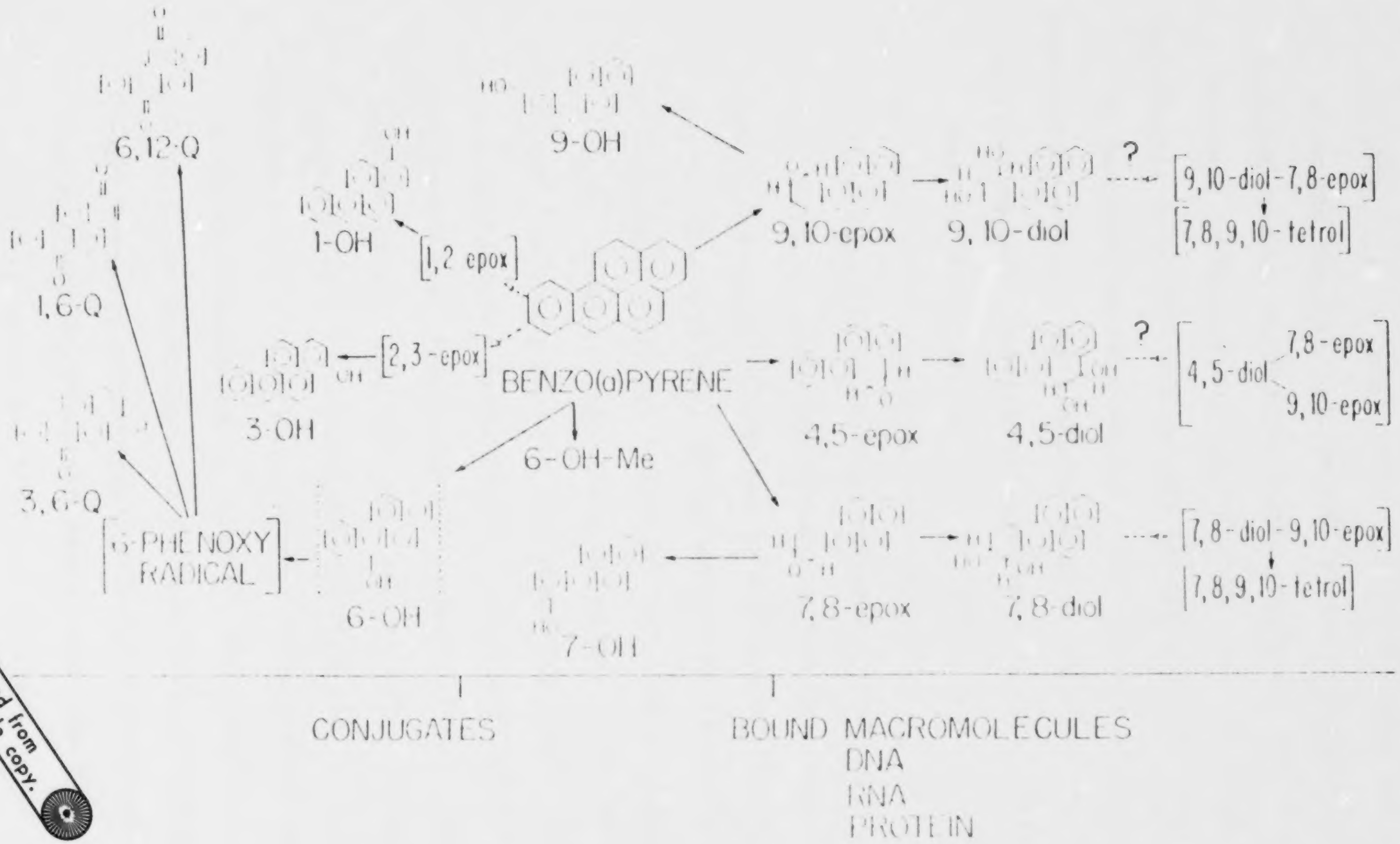
this also appears to be representative of the general mechanism for PAH metabolism.

A discussion of the metabolism of PAH in mammalian species, including man, is best approached by examining in detail the chemical fate of the most representative and well-studied compound in the PAH class, namely BaP. The metabolism of BaP has been extensively studied in rodents, and the results of these investigations provide useful data which can be directly compared to and contrasted wich the results of more limited studies employing human cells and tissues. Therefore, separate discussions are based upon the available experimental evidence regarding PAH metabolism in general, and BaP metabolism in particular, in both animals and man.

Metabolism of PAH in Animals: The metabolites of PAH produced by microsomal enzymes in mammals can arbitrarily be divided into two groups on the basis of solubility. In one group are those metabolites which can be extracted from an aqueous incubation mixture by an organic solvent. This group consists of ring-hydroxylated products such as phenols and dihydrodiols (Selkirk, et al. 1974; Sims, 1970), and hydroxymethyl derivatives of those PAH having aliphatic side chains, such as 7, 12-di-methylbenz(a)anthracene (Boyland and Sims, 1967) and 3-methylcholanthrene (Stoming, et al. 1977; Thakker, et al. 1978). In addition to the hydroxylated metabolites are quinones, produced both enzymaticallv by microsomes and non-enzymatically by air oxidation of phenols. Labile metabolic intermediates such as epoxides can also be found in this fraction (Selkirk, et al. 1971; Sims and Grover, 1974; Selkirk, et al. 1975; Yang, et al. 1978).

In the second group of PAH metabolites are the water soluble products remaining after extraction with an organic solvent. Many of these derivatives are formed by reaction (conjugation) of hydroxylated PAH metabolites with glutathione, glucuronic acid, and sulfate. Enzyme systems involved in the formation of water-soluble metabolites include glutathione S-transferase, UDP-glucuronosyl transferase, and sulfotransferases (Bend, et al. 1976; Jerina and Daly, 1974; Sims and Grover, 1974). Conjugation reactions are believed to represent detoxification mechanisms only, although this group of derivatives has not been rigorously studied.

The metabolite profile of BaP which has recently been expanded and clarified by the use of high pressure liquid chromatography is depicted in Figure 2. This composite diagram shows three groups of positional isomers, three dihydrodiols, three quinones, and several phenols. The major BaP metabolites found in microsomal incubations are 3-hydroxy-BaP, 1-hydroxy-BaP, 7-hydroxy-BaP, and 9-hydroxy-BaP. The BaP-4,5-epoxide has been isolated and identified as a precursor of the BaP-4,5-dihydrodiol. Other studies indicate that epoxides are the precursors of the 7,8-dihydrodiol and 9,10-dihydrodiol as well. Considerable evidence has recently become available which implicates the diol epoxide, 7β,8α-dihydro-7,8-dihydroxybenzo(a)pyrene-9,10α-oxide, as an ultimate carcinogen derived from BaP (Jerina, et al. 1976; Kapitulnik, et al. 1977 and 1978a,b; Levin, et al. 1976a,b; Yang, et al. 1978).



6,12-Q
9-OH
1-OH
1,6-Q
[1,2 epox]
9,10-epox
9, 10-diol
?
[9,10-diol-7,8-epox]
[7,8, 9, 10-tetrol]
[2,3-epox]
3-OH
BENZO(a)PYRENE
4,5-epox
4,5-diol
?
4,5-diol
7,8-epox
9,10-epox
3,6-Q
6-OH-Me
[6-PHENOXY RADICAL]
6-OH
7-OH
7,8-epox
7,8-diol
[7,8-diol-9,10-epox]
[7,8,9,10-tetrol]
CONJUGATES
BOUND MACROMOLECULES
DNA
RNA
PROTEIN

Figure 2. Metabolites of Benzo(a)pyrene

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Since the resonance properties of PAH make ring openings difficult, enzymatic attack in the microsomes functions to open double bonds and add an oxygen-containing moiety, such as a hydroxyl group, to give it more solubility in aqueous media (e.g., urine) and thus facilitate removal from the body. In the formation of metabolic intermediates by oxidation mechanisms, relatively stable PAH are converted to unstable products (i.e., epoxides). Thus, nucleophilic attack of this reactive intermediate, through the formation of a transient carbonium ion, would be greatly enhanced. Arylations of this type are common to many classes of carcinogenic chemicals. Therefore, the microsomal cytochrome P-450-containing MFO system and epoxide hydrase play a critical role in both the metabolic activation and detoxification of many PAH.

Various forms of liver microsomal cytrochrome P-450 can be isolated from animals treated with different enzyme inducers (Wiebel, et al. 1973; Nebert and Felton, 1976; Conney, et al. 1977; Lu, et al. 1978). Moreover, the metabolite profiles of BaP can be qualitatively altered depending on the type of cytochrome P-450 present in the incubation mixture (Lu, et al. 1976; Weibel, et al. 1975). This observation has important implications in considering the carcinogenic action of certain PAH toward tissues from animals of different species, sex, age, nutritional status, and exposure to enzyme-inducing chemicals. Limited evidence is also available indicating that multiple forms of epoxide hydrase exist among animals species, which may also influence the pattern of PAH metabolism with respect to carcinogenic bioactivation (Lu, et al. 1978).

Comparative Metabolism of PAH in Animals and Man: An important consideration in evaluating the health hazards of PAH is whether metabolism in various animal tissues and species is indicative of the pattern of PAH metabolism in the target organs of humans. Moreover, it is essential to determine whether differences occur in the metabolism of PAH by: (a) different tissues in the same animal; and (b) different animals of the same species.

Numerous studies have shown the qualitative and quantitative differences exist in the metabolism of BaP by different tissues and animal species (Sims, 1976; Leber, et al. 1976; Wang, et al. 1976; Pelkonen, 1976; Kimura, et al. 1977; Selkirk, et al. 1976). For the most part, however, interspecies extrapolations of qualitative patterns of PAH metabolism appears to be a valid practice. On the other hand, marked differences in patterns of tissue-specific metabolism may prevent the reliable extrapolation of data from hepatic to extrahepatic (i.e., target organ) tissues. These difference may also exist in human tissues (Conney, et al. 1976).

Freudenthal and coworkers (1978) recently examined the metabolism of BaP by lung microsomes isolated from the rat, rhesus monkey, and man. Metabolite profiles obtained by high pressure liquid chromatography are shown in Figure 3. Their results confirmed previous observations regarding the existence of considerable individual variation in BaP metabolism among samples from the same species. In addition, it was apparent that qualitative and quantitative interspecies variation also existed (Table 17). Nevertheless, the qualitative differences between man and the other animal species were by no means dramatic, and probably do not compromise the validity of extrapolations concerning PAH metabolism.

The metabolite pattern obtainted for BaP in human lymphocytes is similar to that obtained with human liver microsomes (Selkirk, et al. 1975), and human lymphocytes (Booth, et al. 1974). However, in cultured human bronchus (24 hrs.) and pulmonary alveolar macrophages an absence of phenols (i.e., 3-hydroxy-BaP) and paucity of quinones were observed (Autrup, et al. 1978). Instead, a relative abundance of the trans-7,8-diol metabolite of BaP was demonstrated. This result is noteworthy in light of the possiblity that the 7,8-diol is capable of further oxidative metabolism to an ultimate carcinogenic form of BaP. It is not known whether a longer incubation period would have changed the pattern of metabolite formation.

Excretion

There is no direct information available concerning the excretion of PAH in man. Limited inferences can be drawn from animal studies with PAH, however.

As long ago as 1936, researchers recognized that various PAH were excreted primarily through the hepatobiliary system and the feces (Peacock, 1936; Chalmers and Kirby, 1940). However, the rate of disappearance of various PAH from the body, and the principal routes of excretion are influenced

TABLE 17

Metabolite Percentages of BP Metabolites from Rat, Rhesus, and Human Lung Microsomal Assays (Freudenthal, et al. 1978)

	Metabolite percentages (pmoles metabolite/pmoles total metabolites x 100)									
	Rat[a]			Rhesus[b]			Man[b,c]			
Metabolite	1	2	3	1	2	3	1	2	3	4
Pre-9,10					3.0	5.3				
9,10-Diol	9.7	6.3	9.6	2.7	4.6	2.6		7.1	6.0	
A				1.5						
U (B)	4.4	3.4	2.9	6.9		7.7	8.9	3.9	7.5	30.0
4,5-Diol	8.3	9.2	8.3	9.0	9.2	7.7	4.1			
7,8-Diol	5.3	5.2	8.0	4.2	8.6	5.1		15.0	13.3	9.9
1,6-Dione	4.4	7.5	8.3	11.4	14.8	12.8	24.9	11.6	12.6	4.4
3,6-Dione	7.8	8.0	9.9	14.5	16.0	20.5	22.5	13.8	19.2	8.5
6,12-Dione	6.8	8.6	8.6	11.8	8.0	15.3	22.5	18.3	27.4	15.7
9-OH	12.6	11.5	3.5	7.3			5.7	6.2		8.5
3-OH	40.8	40.2	41.1	30.8	35.9	23.1	11.4	24.0	13.9	22.9

[a] Lungs of 5 rats pooled for each group.

[b] Determinations made on lung samples from separate individuals.

[c] With the exception of subject 4, activity determinations were made using microsomes which had been stored at -84°C.

[d] The structural characteristics of unknown, U, may differ between species.

both by structure of the parent compound and the route of administration (Heidelberger and Weiss, 1951; Aitio, 1974). Moreover, the rate of disappearance of a PAH (i.e., benzo(a)-pyrene) from body tissues can be markedly stimulated by prior treatment with inducers of microsomal enzymes (e.g., benzo(a)pyrene, 7, 12-dimethylbenz(a)anthracene, 3-methylcholanthrene, chrysene) (Schlede, et al. 1970a,b). Likewise, it has been shown that inhibitors of microsomal enzyme activity, such as parathion and paraoxon, can decrease the rate of BaP metabolism in certain animal tissues (Weber, et al. 1974). From the available evidence concerning excretion of PAH in animals, it is apparent that extensive bioaccumulation is not likely to occur.

EFFECTS

Acute, Sub-acute, and Chronic Toxicity

The potential for PAH to induce malignant transformation dominates the consideration given to health hazards resulting from exposure. This is because toxic effects are not usually produced by many PAH until doses are well into the carcinogenic range. Although the emphasis on carcinogenicity is certainly justified when dealing with public health issues concerning PAH, one must recognize that non-neoplastic lesions may also result from environmental and occupational contact. Such effects can be seen with low doses of carcinogenic PAH and with those compounds which possess no tumorigenic activity.

As long ago as 1937, investigators knew that carcinogenic PAH, produced systemic toxicity as manifested by an inhibition of body growth in rats and mice (Haddow, et al. 1973). Tissue damage resulting from the administration of various PAH to experimental animals is often widespread and severe, although selective organ destruction may occur (e.g., adrenal necrosis, lymphoid tissue damage). Few investigators, however, have attempted to ascertain the molecular mechanism of PAH-induced cytotoxicity. Nevertheless, current opinion favors the concept that normally proliferating tissues (intestinal epithelium, bone marrow, lymphoid organs, testis) are preferred targets for PAH, and this susceptibility may be due to a specific attack on DNA of cells in the S phase of the mitotic cycle (Philips, et al. 1973). Additional factors which may have an important bearing on the adverse effects resulting from PAH exposure are primary and secondary alterations in enzyme activity and immunologic competence. Moreover, these toxicant-induced changes may play an important role in the eventual induction of neoplasia.

Target organs for the toxic action of PAH are diverse, due partly to extensive distribution in the body and also to the selective attack by these chemicals on proliferating cells. Damage to the hematopoietic and lymphoid systems in experimental animals is a particularly common observation. Yasuhira (1964) described severe degeneration of the thymus and marked reduction in weight of the spleen and mesenteric lymph nodes of CF1 Swiss and C57BL mice given a single intraperitoneal injection of MCA (0.3 to 1.0 mg) between 12 hours and 9 days after birth. Degeneration of young cells in the bone marrow and retardation of thyroid gland development were also noted. Newborn mice were highly susceptible to the toxic effects of MCA, with many animals dying from acute or chronic wasting disease following treatment. Among surviving CF1 mice, numerous thymomas eventually developed; none were evident, however, in C57BL mice despite serious thymic damage.

DMBA is well-known for its effects on the bone marrow and lymphoid tissues. With single feedings (112 or 133 mg/kg B.W.) to female Sprague-Dawley rats, age 50 days, DMBA induced pancytopenia by causing a severe depression of hematopoietic and lymphoid precursors (Cawein and Sydnor, 1968). Maturation arrest occurred at the proerythroblast levels; no injury to the stem cells or the formed elements in the peripheral blood was evident. The fact that only the more rapidly proliferating hematopoietic elements were vulnerable to attack by DMBA led the authors to suggest that inhibition of DNA replication may be involved in the toxicologic response.

Philips and coworkers (1973) provided strong support for the argument that DMBA-induced cytotoxicity is mediated via an interaction with DNA. Female Sprague-Dawley rats receiving 300 mg/kg B.W. DMBA orally and male rats receiving an intravenous injection of 50 mg/kg B.W. DMBA displayed injury to the intestinal epithelium, extreme atrophy of the hematopoietic elements, shrinkage of lymphoid organs, agranulocytosis, lymphopenia, and progressive anemia. Mortality among rats receiving DMBA by gastric intubation (females) was about 65 percent. In rats given 50 mg/kg B.W. DMBA intravenously, incorporation of ^{14}C-labeled thymidine into

DNA of small and large intestine, spleen, bone marrow, cervical lymph nodes, thymus, and testis was significantly inhibited. This inhibition was as high as 90 percent in several organs at six hours, and indicated a strong inhibition of DNA synthesis. Consequently, the authors postulated that DNA in S phase cells is particularly susceptible to DMBA attack. This phenomenon probably applies for other carcinogenic PAH as well.

Another lesion, characteristic of that produced by X-rays, is the severe testicular damage induced by DMBA in rats (Ford and Huggins, 1963). Single intravenous injections of DMBA (0.5 to 2.0 mg) given to adolescent (25 days of age) rats caused transient degenerative changes in the testis which were most evident 38 to 40 days after treatment. Essentially the same effects were produced in adult rats, age 60 days, given DMBA orally (20 mg) and intravenously (5 mg). Lesions of the testes were highly specific and involved destruction of spermatogonia and resting spermatocytes, both of which are the only testicular cells actively synthesizing DNA. Neither the remaining germinal cells nor the interstitial cells were damaged by DMBA. Surprisingly, no testicular damage was produced by single feedings of BaP (100 mg), MCA (105 mg), or 2-acetoaminophenanthrene (40 mg).

For many years researchers have known that the application of carcinogenic polycyclic hydrocarbons to mouse skin leads to the destruction of sebaceous glands, hyperplasia, hyperkeratosis, and even ulceration (Bock, 1964). Sebaceous glands are the skin structures most sensitive to polycyclic hydrocarbons, and assay methods for detection of carcinogens have been based on this effect. Although a good correlation can be obtained between carcinogenic activity and sebaceous gland suppression for many PAH (e.g., MCA, DMBA, BaP, DBA, benz(a)anthracene), such an effect is neither necessary nor sufficient for carcinogenesis. However, workers exposed to PAH-containing materials such as coal tar, mineral oil, and petroleum waxes are known to show chronic dermatitis, hyperkeratoses, etc. (Hueper, 1963; Natl. Acad. Sci. 1972), though the possible significance of these skin disorders to human cancer is not known.

In female animals, ovotoxicity has been reported to result from the administration of PAH. DMBA was shown to cause the destruction of small oocytes and to reduce the numbers of growing and large oocytes after oral administration to mice (Kraup, 1970). More recently a report was published that destruction of primordial oocytes in mice by injection of MCA was correlated with the genetic capability for PAH-induced increases in ovarian aryl hydrocarbon hydroxylase activity (Mattison and Thorgeirsson, 1977). Thus, the ovarian metabolism of PAH and ovotoxicity are apparently linked and are under genetic control.

A toxic reaction which is apparently unique to DMBA is the selective destruction of the adrenal cortex and induction of adrenal apoplexy in rats (Boyland, et al. 1965). Adrenal apoplexy, increased adrenal gland weight, and increased adrenal hemoglobin content were induced in female Sprague-

Dawley rats by a single intragastric dose of 30 mg DMBA. The same amount of adrenal damage could be produced by a 5 mg dose of the principal DMBA oxidative metabolite, 7-hydroxymethol-12-methylbenz(a)anthracene. Other DMBA metabolites produced no adrenal damage, thus indicating that a specific reactive intermediate may be responsible for this phenomenon.

Repeated injections of benz(a)anthracene derivatives to mice and rats have produced gross changes in the lymphoid tissues. Early investigators administered DBA, benz(a)anthracene, and anthracene to mice in weekly subcutaneous injections for 40 weeks (Hoch-Ligeti, 1941). Analysis of lymph glands removed at weekly intervals showed an increase of reticulum (stem) cells and an accumulation of iron in all treatment groups. Lymphoid cells were reduced and lymph sinuses dilated in all groups, although these effects were more common in mice receiving DBA. The weights of the spleens in mice treated with DBA were significantly reduced in comparison to controls and those animals receiving benz(a)anthracene or anthracene.

A more detailed study on the effects of repeated administration of DBA on lymph nodes of male rats was reported in 1944 (Lasnitzki and Woodhouse, 1944). Subcutaneous injections given five times weekly for several weeks caused normal lymph nodes to undergo hemolymphatic changes. These changes are characterized by the presence of extravascular red blood cells in the lymph spaces and the presence of large pigmented cells. These changes were not observed by Hoch-Ligeti (1941) in mice, but could be produced in rats by BaP and MCA in addition to DBA. The non-carcinogen, anthracene, on the other hand, did not produce as dramatic a change in the lymph nodes of rats.

In light of the concern over PAH-induced neoplasms of the respiratory tract, an understanding of early patholcgical alterations and pre-neoplastic lesions in this tissue has particular significance.

In a study conducted by Reznik-Schuller and Mohr (1974), BaP-induced damage to the bronchial epithelium of Syrian golden hamsters was examined in detail using semithin (1 µm) tissue sections. Animals were treated intratracheally with 0.63 mg BaP (total dose) dispersed in a solution of saline, dodecylsulfate, Tris-HCl, and EDTA once weekly for life. Animals were serially sacrificed at weekly intervals following the first month of treatment, and semithin sections of the bronchi were examined microscopically. In the first animals sacrificed, minimal focal cell proliferation in the area of the basement membrane was evident in the bronchial epithelium. By 7 weeks, cytoplasmic vacuolization of both goblet and ciliated cells had occurred. Epithelial and basal cell proliferation continued for several weeks and led to the formation of three- to four-layered hyperplastic regions by the 11th week. Epithelial cells began to penetrate through the basement membrane by the 12th week, and within 2 or more weeks the bronchial epithelium began to continuously grow into the surrounding lung tissues. Microscopic bronchogenic adenomata had developed by the 20th week. These tumors consisted primarily of ciliated cells and goblet cells, with only a few basal cells present. The apparently small amount of basal cell proliferation may have been the reason why squamous metaplasia was not observed by the time the experiment had ended after 21 weeks. Squamous metaplasia and keratinization were found in the trachea, but not in the bronchi, after 21 weeks of treatment. Although these investigators found no increase in the number of alveolar macrophages, others have reported numerous alveolar macrophage responses in BaP-treated hamsters as well as focal areas of accumulated macrophages containing a yellow pigment having unknown biological significance (Henry, et al. 1973; Saffiotti, et al. 1968).

Epithelial proliferation and cell hyperplasia in the absence of necrosis and/or marked inflammation is a common observation in the tracheobronchial mucosa of animals directly exposed to carcinogenic PAH. This phenomenon was shown with repeated exposures of DMBA, BaP, and dibenzo(a,i)pyrene in hamsters (Reznik-Schuller and Mohr, 1974; Saffiotti, et al. 1968; Stenback and Sellakumar, 1974a,b).

Numerous investigators have demonstrated that carcinogenic PAH can produce an immunosuppressive effect. This effect was first observed by Malmgren, et al. (1952) using high doses of MCA and DB(a,h)A in mice. Subsequent studies established that single carcinogenic doses of MCA, DMBA, and BaP caused a prolonged depression of the immune response to sheep red blood cells (Stjernsward, 1966, 1969). Noncarcinogenic hydrocarbons such as benzo(e)pyrene and anthracene reportedly had no immunosuppressive activity. In a recent review on immunosuppression and chemical carcinogenesis, substantial evidence was presented to indicate that the degree of immunosuppression was correlated with carcinogenic potency for PAH (Baldwin, 1973). Both cell-mediated and humoral immune reactions are affected by PAH.

Synergism and/or Antagonism

It is well-known that the development of PAH-induced tumors in epithelial and non-epithelial tissues can be altered by: (1) components in the diet, (2) inducers and inhibitors of microsomal enzymes, (3) other co-administered noncarcinogenic or weakly carcinogenic chemicals, and (4) the vehicle used to deliver a carcinogenic PAH to experimental animals. These factors tend to complicate the extrapolation of animal dose-response data to human situations. On the other hand, these observations in animals reinforce the belief that similar interactions occur with regard to the action of PAH in humans.

Early studies conducted by Falk and coworkers (1964) indicated that the carcinogenic effect of BaP on subcutaneous injection in mice could be markedly inhibited by the simultaneous administration of various noncarcinogenic PAH. Similarly, they showed that neutral extracts of particulate air pollutant fractions also produced inhibitory effects on BaP-induced tumorigenesis. However, when Pfeiffer (1973, 1977) conducted similar studies with BaP and DBA in the presence of 10 noncarcinogenic PAH, no inhibitory effect was evident. Moreover, an increased tumor yield resulted from injection of mixtures containing increasing amounts of the components. This effect, however, was less dramatic than if BaP were administered alone, and it paralleled the dose-response curve for DBA acting singly.

Many studies on cocarcinogenesis have been concerned with the identification of tumor accelerating substances present in cigarette smoke. These compounds are generally tested for cocarcinogenic activity by repeated application to mouse skin together with low doses of BaP. A positive response would be obtained in cases where the tumor yield of the combination exceeds that produced by either agent alone at the same doses. Van Duuren and coworkers (1973, 1976) established that a pronounced cocarcinogenic effect could be obtained with catechol and the noncarcinogens, pyrene, BeP, and benzo(g,h,i)perylene. Doses of 12, 15, 21, and 2,000 μg of these compounds, respectively, were applied three times a week for 52 weeks to female ICR/Ha Swiss mice. Each animal also received 5 μg of BaP in 0.1 ml acetone with each dose of test substance. Although phenol has been regarded as a tumor-promotor in the two-stage carcinogenesis sytem (Van Duuren, 1976), this compound has a slight inhibitory effect on BaP carcinogenesis when administered in combination. These results, therefore, indicated that tumor-promotors and cocarcinogens may not have the same mode of action, and that the two terms should not be used interchangeably. Other PAH (e.g., fluoranthene, pyrene, pyrogallol) also possess cocarcinogenic activity but have no tumor-promoting activity (Van Duuren, 1976). Additional studies by Schmeltz, et al. (1978) established that most of the naphthalenes found in cigarette smoke have an inhibitory effect on skin tumorigenesis (250 μg, three times a week) as induced by BaP (3 μg, three times a week). On the other hand, several of the alkylnaphthalenes tested (dimethyl-, trimethyl-, tetramethyl-) enhanced the carcinogenic activity of BaP on mouse skin.

Numerous investigators have shown that antioxidants are effective inhibitors of PAH-induced tumor development. This action has been demonstrated with selenium (Shamberger, 1970; Shamberger and Rudolph, 1966; Riley, 1969), dl-α-tocopherol (Vitamin E) (Shamberger, 1970; Shamberger and Rudolph, 1966), and ascorbic acid (Shamberger, 1972) in mice treated with DMBA and croton oil. The carcinogenic action of MCA has been reduced by tocopherol-rich diets in rats and mice (Jaffe, 1946; Haber and Wissler, 1962). The antioxidant food additives butylated hydroxytoluene (BHT), ethoxyquin, and butylated hydroxyanisole (BHA) have inhibited lung, breast, and gastric tumor formation induced in rats and mice by various carcinogens in the diet (Wattenberg, 1972, 1973; Wattenberg, et al. 1976). The sulfur-containing antioxidants disulfuram, dimethyldithiocarbamate, and benzyl thiocyanate, inhibited DMBA-induced mammary cancer in rats when they were added to the diet; in the mouse, disulfuram prevented the formation of forestomach tumors induced by BaP in the diet, but had no effect on BaP-induced pulmonary adenoma (Wattenberg, 1974). The agricultural herbicide, maleic hydrazide, and its precursor, maleic anhydride, can inhibit the initiating activity of DMBA in the mouse skin two-stage carcinogenesis system (Akin, 1976).

Rahimtula and coworkers (1977) examined the abilities of several antioxidants to affect BaP hydroxylation by rat liver microsomal mixed-function oxidases. Their results indicated that antioxidants can markedly inhibit BaP hydroxylation by an apparently direct action on microsomal oxidation mechanisms. Furthermore, all of the antioxidants tested reduced the bacterial mutagenicity of BaP in the presence of rat liver microsomes and cofactors. The authors suggested that antioxidants may exert their protective effect *in vivo* by inhibiting the formation of carcinogenic intermediates from PAH. This conclusion, however, seems to conflict with data indicating that inducers of increased BaP hydroxylase activity can also inhibit tumor formation (Wattenberg and Leong, 1970). However, flavones are also inhibitors of BaP metabolism *in vitro*, thereby indicating that their specific effects depend upon how and where they are used. These investigators found that several synthetic and naturally occurring flavones when incorporated in the diet (3 to 5 mg/g) or applied to the skin caused a profound increase in BaP hydroxylase activity in the small intestine and skin, respectively. In addition, pulmonary adenoma formation resulting from oral administration of BaP was totally prevented, and skin tumors initiated by BaP application to mice were significantly reduced (>50 percent) by treatment with the synthetic flavone, β-naphthoflavone. Pulmonary tumor formation was also reduced 50 percent by incorporation of the naturally occurring flavone, quercetin pentamethyl ether, into the diet. Sullivan and coworkers (1978) recently demonstrated that BHA, BHT, phenothiazine, phenothiazine methosulfate, and ethoxyquin can all reduce the quantitative yield of BaP metabolites in incubations with rat liver microsomes. The possibility that only specific components of the drug metabolizing enzyme system may be induced by antioxidants has not been fully explored.

In addition to flavones, other naturally occurring compounds have exhibited protective effects against PAH-induced tumor formation. Vitamin A has clearly been shown to play a role in reducing carcinogen-induced tumors (Nettesheim, et al. 1975; Cone and Nettesheim, 1973; Chu and Malmgren, 1965; Smith, et al. 1975). Nettesheim and Williams (1976) recently examined whether inadequate vitamin A consumption may predispose individuals to carcinogenesis, or whether increased vitamin A intake exerts a protective effect against neoplasia. They found that a diet deficient in vitamin A increased the formation of MCA-induced metaplastic lung nodules in female Fisher 344 rats, even though adequate amounts of the vitamin were stored in the liver. On the other hand, moderate amounts of the vitamin A added to the diet markedly reduced the development of MCA-induced lesions of the lung. High doses of the vitamin given intragastrically provided no additional protection, however.

Further studies on naturally occurring antineoplastic compounds were recently reported by Wattenberg (1977). Benzyl isothiocyanate and phenethyl isothiocyanate, both found in cruciferous plants such as cabbage, brussel sprouts, cauliflower, etc., inhibited DMBA-induced mammary cancer in Sprague-Dawley rats. When added to the diet together with DMBA, these compounds inhibited the development of forestomach tumors and pulmonary adenomas in female ICR/Ha mice. Similar anticarcinogenic actions were obtained when BaP was incorporated into the diet. These results lead to interesting speculation regarding the role and importance of diet in human susceptibility to environmental carcinogens. In cases where dietary consituents can alter the metabolism of xenobiotics such as PAH, then the anticarcinogenic effect may result from an alteration of steady state levels of activated versus detoxified metabolites.

Studies have shown that not only can specific substances in the diet affect the response to carcinogens, but decreased protein content in the diet may also decrease the activation of carcinogens (Czygan, et al. 1974). The feeding of protein-deficient diets to male mice decreased liver weights, and reduced cytochrome P-450 content in the total liver. Diets deficient in both protein and choline produced even further reductions in liver weight and cytochrome P-450 content. Liver microsomes isolated from these animals displayed a decreased ability to activate dimethylnitrosamine to a mutagen (in the Ames Salmonella test system), which paralleled the reduction in cytochrome P-450 content produced by the diet. Conversely, the inactivation of the direct-acting (ultimate) carcinogen N-methyl-N'-nitro-N-nitrosoguanidine was reduced in liver microsomes from mice receiving a protein-deficient diet.

In humans fed charcoal-broiled beef, the metabolism of the drug phenacetin was enhanced; in pregnant rats a similar diet stimulated the activity of AHH in the placenta and liver (Conney, et al. 1977a,b). Further studies showed that high-protein diets enhanced the metabolism of antipyrene and theophylline in man, while a high-carbohydrate diet depressed the rate of metabolism of these drugs. Additional agents in man's environment which inhibit AHH activity include certain organophosphate pesticides, piperonyl butoxide, carbon tetrachloride, ozone, carbon monoxide, nickel carbonyl, and nickel, tin, cobalt, and other metals (Conney, et al. 1977a,b). It is not known whether exposure of humans to these agents may affect susceptiblity to cancer formation (see Criterion Formulation section).

Teratogenicity

No information is available concerning the possible teratogenic effects of PAH in man. Furthermore, only limited data are available regarding the teratogenic effects of PAH in experimental animals.

BaP had little effect on fertility or the developing embryo in several mammalian and non-mammalian species (Rigdon and Rennels, 1964; Rigdon and Neal, 1965). On the other hand, DMBA and its hydroxymethyl derivatives apparently are teratogenic in the rat (Currie, et al. 1970; Bird, et al. 1970). However, DMBA is not generally regarded as an environmental contaminant.

Mutagenicity

No reliable way presently exists to measure whether PAH may induce heritable mutations in humans. However, the concept that carcinogenesis is an expression of an alteration in the genetic material of a cell (i.e., somatic mutation) implies that a formal relationship exists between mutagenesis and carcinogenesis (Nery, 1976; Miller, 1978). The results obtained with several *in vitro* mutagenesis test systems, particularly the Ames *Salmonella typhimurium* assay, support the belief that most carcinogenic chemicals are mutagenic as well. For PAH, the Ames assay has been very effective in detecting those parent structures and their biotransformation products which possess carcinogenic activity (McCann, et al. 1975; Teranishi, et al. 1975; McCann and Ames, 1976; Sugimura, et al. 1976; Wislocki, et al. 1976b; Wood, et al. 1976a; Tokiwa, et al. 1977; Brookes, 1977). The Ames assay, however, may not be 100 percent effective in detecting all PAH carcinogens.

The availability of *Salmonella typhimurium* strains for the detection of chemically induced mutations and the use of microsomal preparation to provide metabolic activation, has made possible an investigation of the mechanisms of PAH-induced mutagenesis. In particular, an exhaustive survey of the mutagenicity of all the possible oxidative metabolites of BaP has helped to confirm the belief that diol epoxide intermediates are the ultimate mutagens/carcinogens derived from PAH (Jerina, et al. 1976; Wood, et al. 1976a,b; Wislocki, et al. 1976a,b; Thakker, et al. 1976; Levin, et al. 1977a,b). These results are summarized in Table 18.

Further examination of the mutagenic activity of PAH and their derivatives has been conducted in mammalian cell culture systems. These systems operate with concentrations of test compounds which are lower than those used in the Ames assay. This work has been conducted primarily with Chinese hamster cell lines, either V79 cells derived from male lung tissue or CHO cells derived from the ovary. These cells, however, do not possess a microsomal enzyme system and thus co-cultivation with lethally irradiated rodent embryo cells which retain metabolic activity is required for testing of PAH.

Using this system, Huberman and Sachs (1974, 1976) demonstrated that a number of carcinogenic PAH produced forward mutations involving three genetic markers: (1) ouabain resistance; (2) temperature sensitivity; and (3) 8-azaguanine resistance. Noncarcinogenic PAH such as BeP, phenanthrene, and pyrene were not mutagenic. In addition, studies by Huberman indicated that a correlation could be shown between the degree of carcinogenicity and the frequency of induced somatic mutations (Huberman, et al. 1977). The demonstration that covalent binding of carcinogenic PAH with DNA of V79 cells was the same as occurs in vivo further strengthed the argument that genetic interaction (i.e., somatic mutation or gene depression) may be involved in tumor formation (Newbold, et al. 1977).

The use of Chinese hamster V79 cells to test the mutagenicity of BaP metabolites has contributed significantly

TABLE 18

Comparison of Inherent Mutagenic Activity of Thirty BaP Derivatives in *Salmonella typhimurium* TA98 and in Chinese Hamster V79 Cells[a] (Jerina, et al. 1976)

Compound[b]	Relative % activity	
	Strain TA98	V79
Diol epoxide-1	100	40
Diol epoxide-2	35	100
H_4 9,10-epoxide	95	40
H_4 7,8-epoxide	10	0.2
BaP 4,5-oxide	20	1
BaP 7,8-oxide	1	<0.1
BaP 9,10-oxide	1	<0.1
BaP 11,12-oxide	0.5	1
6-HOBaP	5	0.3
12-HOBaP	1.5	<0.1
1-HOBaP	0.5	0.1
3-HOBap	0.5	<0.1
2-, 4-, 5-, 7-, 8-, 9-, 10-, 11-HOBaP	<0.1	<0.1
BaP 1,6-, 3,6-, 6,12-, 4,5-, 11,12-quinone	<0.1	<0.1
BaP 4,5-, 7,8-, 9,10-, 11,12-dihydrodiol	<0.1	<0.1
BaP	<0.1	<0.1

[a] The relative percent mutagenic activities are approximations since the data were compiled from several separate studies conducted at different times. In some experiments, BaP 7,8-dihydrodiol was 0.1 to 0.4% as active as diol epoxide-2 in V79 cells.

[b] Abbreviations used: BaP, benzo(a)pyrene; 1-HOBaP, 1-hydroxybenzo(a)pyrene; 2- to 12-HOBaP, other BaP phenols; BaP 1,6-quinone, benzo(a)pyrene 1,6-quinone; BaP 3,6-quinone, BaP 4,5-quinone, BaP 6,12-quinone, and BaP 11,12-quinone, other BaP quinones; BaP 4,5-dihydrodiol, trans-4,5-dihydroxy-4,5-dihydrobenzo(a)pyrene; BaP 7,8-, 9,10- and 11,12-dihydrodiol, other dihydrodiols of BaP; BaP 4,5-oxide, benzo(a)pyrene 4,5-oxide; BaP 7,8-, 9,10-, and 11,12-oxide, other BaP oxides; diol epoxide-1(±)-7β,8α-dihydroxy-9β,10β-epoxy-7,8,9.10-tetrahydro BaP; diol epoxide-2, (±)-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydro BaP; H_4 9,10-epoxide, 9,10-epoxy-7,8,9,10-tetrahydro BaP; H_4 7,8-epoxide, 7,9-epoxy-7,8,9,10-tetrahydro BaP.

to an understanding of the molecular action of PAH (Huberman, et al. 1977, 1976a,b; Maleveille, et al. 1975; Newbold and Brookes, 1976; Jerina, et al. 1976). Comparison of the mutagenic activities of the optically pure (+) and (-)-enantiomers of BaP 7,8-dihydrodiol revealed that, in the presence of a metabolic activating system, the (-)trans, 7,8-dihydrodiol was the most active mutagen (Huberman, et al. 1977). These results are consistent with the fact that the (-)trans 7,8-dihydrodiol is the only BaP enantiomer by rat liver microsomes (Yang, et al. 1977), and that it is highly carcinogenic to newborn mice (Kapitulnik, et al. 1978a,b). Because the (-)trans 7,8-dihydrodiol had no mutagenic activity in the absence of enzymes required for PAH metabolism, it was apparent that the BaP 7,8-diol-9,10-epoxide which is derived from this intermediate is an ultimate mutagen/carcinogen. Studies by Wood, et al. (1977) on the mutagenicity to V79 cells by the four optically pure enantiomers of the BaP 7,8-diol-9-10-epoxides supported this belief. None of the triols and tetrols which are derived from BaP diol epoxides were mutagenic to V70 cells, and thus represent probable detoxification products (Huberman, et al. 1977).

The current belief that neoplastic transformation may arise from a chemically induced somatic mutation was made even more convincing by the recent studies of Huberman and coworkers (1976b). They demonstrated for the first time that BaP and BaP 7,8-dihydrodiol can induce both neoplastic transformation and mutagenesis (ouabain resistance) in the same culture of normal diploid hamster embryo cells.

In further adaptation of the cell-mediated mutagenesis system, V79 cells are metabolically activated by rat liver homogenates containing microsomes and cofactors (Krahn and Heidelberger, 1977). The mutagenic activity of BaP, MCA, DMBA, and benz(a)anthracene in this system showed a limited correlation with their respective carcinogenic potencies. It should be noted, however, that the selection of a particular activating system (i.e., microsomes vs. feeder cells) may have a significant influence on the test results.

The analysis of chromosomal aberrations and sister chromatid exchanges (SCE's) is often recommended as a screening technique for potential mutagens and carcinogens. Several investigators have examined the effects of PAH on the chromosomes of mammalian cells. Early studies indicated that variations in chromosome number and structure may accompany tumors induced by BaP, MCA, and DMBA in the rat, mouse, and hamster (Kato, et al. 1975). However, in cultured human leukocytes exposed to DMBA, chromosome damage was not the same as that produced in hamster cells. Although it is argued that chromosome changes in PAH-induced tumors are all specific (Levan and Levan, 1975; Ahlstrom, 1974), others (Popescu, et al. 1976; Nery, 1976) claim that detectable chromosome changes are not specific for the carcinogenic agent nor are they a prerequisite for neoplastic growth. Moreover, an increased rate of SCE's can be produced by BaP in cultured human lymphocytes (Rudiger, et al. 1976;

Schönwald, et al. 1977) but this increase is not correlated with different rates of BaP metabolism (Rudiger, et al. 1976), a surprising result in light of the known importance of metabolic activation for BaP mutagenicity. BaP-induced SCE's rates did not differ between lymphocytes taken from normal humans and those from patients with lung cancer (Schönwald, et al. 1977). In recent studies with cultured Chinese hamster cells exposed to DMBA, BaP, and MCA, none of the chemicals produced chromosome breaks and only DMBA could successfully induce SCE's (Abe and Sasaki, 1977). Although it cannot be denied that PAH cause chromosome damage, it is not clear whether this effect may represent an epigenetic phenomenon which is merely secondary to mutagenesis and neoplastic transformation. Furthermore, in cases where a chemically induced mutation is "silent" (i.e., neutral amino acid substitution), there is no reason to believe that detectable chromosome damage should occur.

In recent comparisons of three cytogenetic tests, (1) induction of chromosome aberrations, (2) induction of micronuclei, and (3) in vivo induction of sister chromatid exchanges, the last test proved to be the most sensitive with carcinogenic polycyclic hydrocarbons (Bayer, 1978). Since positive results were also obtained with phenanthrene, however, the usefulness of sister chromatid exchange as a screening technique for carcinogen detection is limited. BaP was positive in the sister chromatid exchange test, weakly active in the chromosome aberration test, and negative in the micro-nucleus test. On the other hand, DMBA was clearly positive in all three tests. The conclusion was that cytological tests do not provide reliable correlations with all carcinogens tested and thus cannot be used alone in mutagenicity/carcinogenicity evaluations.

Damage to the genome resulting from chemical insult can theoretically also be detected by examining DNA repair (Stich and Laishes, 1973). The suggestion that DNA repair is applicable as a screening procedure for evaluating potential chemical mutagens is based on the assumption that the level of DNA repair synthesis in a cell reflects the extent of DNA damage produced by a chemical. Indeed, unscheduled incorporation of ^{3}H-thymidine into nuclear DNA of normal human cells exposed to epoxides of benz(a)anthracene and MCA has been observed (Stich and Laishes, 1973). However, since a metabolic activation system was not present in this system, the parent hydrocarbons showed no activity. More recent studies confirmed that K-region epoxides of BaP, DMBA, and DBahA caused DNA damage in human skin fibroblasts which was repaired with the same system used for repairing lesions induced by ultraviolet radiation (Maher, et al. 1977). As would be expected, the parent hydrocarbons exerted no effect. More important, results were obtained which indicated that the DNA repair process itself does not induce mutations, but rather that mutagenesis occurs before the DNA lesion can be excised.

DNA repair synthesis in human fibroblasts (Regan, et al. 1978; Stich, et al. 1975, 1976; San and Stich, 1975), rat liver cells (Williams, 1976), and Chinese hamster V79 cells (Swenberg, et al. 1976) has been successfully used for the detection of chemical carcinogens, including numerous PAH. However, the percentage of carcinogens giving positive results for DNA repair is considerably less than in the cell transformation or microbial mutagenesis assays. Nevertheless, tests with human skin fibroblasts showed that DNA repair synthesis results from exposure to BaP 7,8-diol-9,10-epoxides, whereas BaP 4,5-, 9,10-, and 11,12-oxides did not produce DNA damage which was repairable by the ultraviolet excision repair system (Regan, et al. 1978). These results support the concept that diol epoxide metabolites of PAH are ultimate mutagens.

Tumors induced in vivo by PAH are commonly associated with chromosome abnormalities in the neoplastic cells. In particular, sarcomas induced by DMBA, MCA, and BaP in the rat display karyotype variations which were reportedly nonrandom and distinctly different from sarcomas induced by Rous sarcoma virus (Levan and Levan, 1975; Mitelman, et al. 1972). The chromosome patterns of DMBA-induced sarcomas were found to be identical with those observed in primary rat leukemias (Mitelman and Levan, 1972) and in primary carcinomas of the auricular skin (Ahlstrom, 1974) induced by DMBA.

Considerable evidence is also available to indicate that chromosome alterations in PAH-induced tumors in vivo are not consistent either in frequency or in pattern. DMBA-induced tumors (fibrosarcoma, squamous carcinoma, lymphosarcoma) of the uterine cervix in ICR mice revealed various karyotypic compositions (Joneja and Coulson, 1973; Joneja, et al. 1971). These tumors displayed diploid, aneuploid, tetraploid, and octaploid chromosome constitutions. Tumors induced in mice with MCA and dibenzo(a,i)pyrene also showed a wide variation in chromosome constitution (Biedler, et al. 1961; Hellstrom, 1959). Mice treated with 30 µg DMBA, a dose sufficient to produce a 100 percent incidence of thymic lymphomas, did not reveal an excess of chromosome abnormalities in bone marrow or thymus (Ottonen and Ball, 1973). Even at higher doses (60 µg DMBA), the incidence of abnormal chromosomes did not significantly differ from controls. Subcutaneous tumors in Syrian hamsters induced by single injections of BaP (0.1 µg) or DMBA (0.1 mg), and cultured cell populations derived from these tumors, failed to reveal common karyotypic changes (DiPaolo, et al. 1971). Tumor cells had subdiploid, diploid, and hypotetraploid chromosome constitutions; further karyotype rearrangements occurred with subsequent growth _in vitro_.

In humans, the presence of the "Philadelphia" chromosome in myeloid leukemia appears to be the only example of a human chromosome abnormality which is tumor-specific (Nowell and Hungerford, 1960). In PAH-induced experimental tumors, lymphatic leukemia in mice produced by DMBA also displays consistent chromosome abnormalities (Joneja and Coulson, 1973). Beyond this common feature, convincing data have not been presented to indicate that somatic cells exposed to PAH may suffer characteristic or reproducible damage to the genome. Instead, random karyotypic mutants of transformed cells are thought to be selected in response to growth pressures in the host environment (e.g., tissue necrosis, infection, anoxia, lack of nutrition) (Joneja and Coulson, 1973).

Evidence has not been encountered in the published literature concerning the likelihood of PAH-induced somatic mutation in the absence of neoplastic transformation.

Carcinogenicity

Animal data: Numerous polycyclic aromatic compounds are distinctive in their ability to produce tumors in skin and most epthelial tissues of practically all species tested. Malignancies are often induced by acute exposures to microgram quantities of PAH. Latency periods can be short (four to eight weeks) and the tumors produced may resemble human carcinomas. Carcinogenesis studies involving PAH have historically involved primarily effects on the skin or lungs. In addition, subcutaneous or intramuscular injections are frequently employed to produce sarcomas at the injection site. Ingestion has not been a preferred route of administration for the bioassay of PAH.

Concern over potential human cancer risk posed by PAH present in the atmosphere stems from studies demonstrating that crude extracts of airborne particulate matter can be carcinogenic to animals (Hoffmann and Wynder, 1976; Wynder and Hoffman, 1965; Hueper, et al. 1962; Kotin, et al. 1954). Fractions soluble in benzene or benzene-methanol produced tumors in mice by skin painting or subcutaneous injection. Both the aromatic and oxygenated neutral subfractions were active as complete carcinogens, and indicated the presence of numerous carcinogenic materials, including non-PAH. Since the carcinogenicity of the total organic particulates and aromatic neutral subfractions could be explained only partly by the presence of BaP, its usefulness as a measure of carcinogenic risk from air pollution may be limited.

From investigations in which polycyclic carcinogens were painted on the skin of mice has emerged the two-stage theory of skin carcinogenesis (Van Duuren, 1969,1976). The first stage, initiation, results from the ability of a carcinogen to effect a permanent change within a cell or cell population following a single application. The measure of carcinogenic potency is often regarded as the capacity for tumor initiation. However, some weak or inactive complete carcinogens can be active as tumor initiators (e.g., dibenz(a,c)anthracene, 1-methylchrysene, benz(a)anthracene). The second stage, promotion, is a prolonged process which does not necessarily require the presence of a carcinogen, but nevertheless a chemical stimulus must be supplied (e.g., by croton oil). A complete carcinogen is one which, if applied in sufficient quantity, can supply both initiating and promoting stimuli (e.g., DMBA, BaP). The formation of skin tumors by polycyclic hydrocarbons may also be influenced by inhibitors and accelerators (cocarcinogens), thus complicating the interpretation of experimental data.

The tumorigenic effects of PAH when applied to the skin of animals have been known for decades. Iball (1939) collected the results of a series of experiments to arrive at a method for comparing the carcinogenic potencies of various polycyclic aromatic chemicals. His results, presented in Table 19, express tumorigenic potency in mouse skin as the ratio of percent tumor incidence to the average latency period. This expression, commonly referred to as the Iball index, is still used as a means of comparing the relative activity of carcinogens. An important data compilation on agents tested for carcinogenicity has more recently been published by the U.S. Public Health Service (Publication No. 149) which lists the results of tests on hundreds of chemicals in numerous animals including rodent, avian, and amphibian species.

Experimental models for respiratory carcinogenesis have major limitations in that the delivery of carcinogens to the tracheobronchial tree in measured amounts and their adequate retention at the target tissue are poorly controlled. Therefore, the conduct of dose-response studies on lung tumor induction has been seriously hampered. Moreover, the possible relevance of the two-stage theory of carcinogenesis to lung cancer has not been clearly established. Many of the bioassay data on PAH-induced lung cancer have been derived from animal model systems employing various modes of administration (inhalation, intratracheal instillation, intravenous injection), and the use of carrier particles (e.g., ferric oxide) for the delivery of the carcinogen to the bronchial epithelium. Thus, the results obtained from these studies cannot always be directly compared. The most commonly employed method for the study of PAH-induced

TABLE 19

Carcinogenic Compounds in Descending Order of Potency (Iball, 1939)

Compound	Number of mice alive when first tumor appears	Number of tumors	Percentage of tumors	Papilloma	Epithelioma	Average latent period (b)	Index (A/B x 100)
1. 7,12-Dimethylbenz(a)anthracene	20	13	65	6	7	43	151
2. 3-Methylcholanthrene (a)	18	18	100	1	17	99	101
3. 3-Methylcholanthrene (b)	8	5	62.5	0	5	151	41
4. 3-Methylcholanthrene (a and b added together)	26	23	88.5	1	22	109	80
5. Benzo(a)pyrene (from pitch)	10	10	100	2	8	127	79
6. Benzo(a)pyrene (synthetic)	9	7	78	2	5	109	72
7. Benzo(a)pyrene (5 and 6 added together)	19	17	89.5	4	13	119	75
8. Cholanthrene	49	28	57	5	23	112	51
9. 5,6-cycloPenteno-benz(a)anthracene	14	13	93	1	12	194	48
10. 2-Methyl-benzo(c)phenanthrene	16	12	75	5	7	155	48
11. 10-Methyl-benz(a)anthracene	18	12	66.5	2	10	147	45
12. 5,6-Dimethyl-benz(a)anthracene	19	16	84	0	16	220	38
13. 6-isoPropyl-benz(a)anthracene	15	11	73.5	1	10	204	36
14. Dibenzo(c,g)carbazole	19	9	47.5	4	5	143	33
15. Dibenzo(a,h)pyrene	17	10	59	0	10	205	29
16. 5-Methyl-benz(a)anthracene	8	7	87.5	2	5	317	28
17. 5-Ethyl-benz(a)anthracene	9	7	77.5	2	5	285	27
18. Dibenz(a,h)anthracene	65	41	63	8	33	239	26
19. Benzo(c)phenanthrene	18	12	67	5	7	387	17
20. Dibenzo(a,g)carbazole	9	4	44.5	1	3	263	17
21. 5-n-Propyl-benz(a)anthracene	20	6	30	3	3	192	16
22. Dibenz(c,h)acridine	28	11	39.3	2	9	357	11
23. 3-Methyl-dibenz(a,h)anthracene	25	7	28	1	6	325	9
24. Dibenz(a,h)acridine	25	6	24	2	4	350	7
	Totals	305		60	245		

lung cancer involves intratracheal instillation of test material in the Syrian golden hamster.

Following the identification of the first carcinogenic hydrocarbon from soot (BaP) an intensive effort was mounted to isolate the various active components of carcinogenic tars (Int. Agency Res. Cancer, 1973). From the earliest studies conducted, the realization emerged that carcinogenic PAH are structurally derived from the simple angular phenanthrene nucleus (Arcos and Argus, 1974). However, unsubstituted PAH with less than four condensed rings that have been tested have not shown tumorigenic activity. Furthermore, of the six possible arrangements with four benzene rings, only two of these compounds are active: benzo(c)phenanthrene and benz(a)anthracene. The unsubstituted penta- and hexacyclic aromatic hydrocarbons are clearly the most potent of the series. These include BaP, DBahA, dibenzo(a,h)pyrene, dibenzo(a,i)pyrene, dibenzo(a,l)pyrene, dibenzo(a,e)pyrene, benzo(b)fluoranthene, and benzo(j)fluoranthene. Somewhat less potent as carcinogens are the dibenzanthracenes and dibenzophenanthrenes. Only a few heptacyclic hydrocarbons show carcinogenic activity. These include phenanthro(2',3':3,4')-pyrene, peropyrene, and dibenzo(h,rst)pentaphene. Beyond seven unsubstituted aromatic rings, there are very few known carcinogenic hydrocarbons. However, many physico-chemical and enzymatic parameters must be dealt with in respect to carcinogenic PAH. Factors such as solubility and intracellular localization to achieve metabolic activation are likely to be important determinants of the true carcinogenicity of a particular PAH.

Among the unsubstituted polycyclic hydrocarbons containing a nonaromatic ring, a number of active carcinogens are known. The most prominent examples of this type of compound are cholanthrene, 11,12-ace-benz(a)anthracene, 8,9-cyclopentano-benz(a)anthracene, 6,7-ace-benz(a)anthracene, acenaphthanthracene, 1,2,5,6-tetrahydrobenzo(j)cyclopent(f,g)aceanthrylene, and "angular" steranthrene. All of these compounds retain an intact conjugated phananthrene segment.

The addition of alkyl substituents in certain positions in the ring system of a fully aromatic hydrocarbon will often confer carcinogenic activity or dramatically enhance existing carcinogenic potency. In this regard, Arcos and Argus (1974) noted that monomethyl substitution of benz(a)anthracene can lead to strong carcinogenicity in mice, with potency depending on the position of substitution in the decreasing order, $7 > 6 > 8 \cong 12 > 9$. A further enhancement of carcinogenic activity is produced by appropriate dimethyl substitution of benz(a)anthracene. Active compounds are produced by 6,8-dimethyl-, 8,9-dimethyl-, 8,12-dimethyl-, 7,8-dimethyl-, and 7,12-dimethyl-substitution. The latter compound is among the most potent POM carcinogens known, although it has not been shown as a product of fossil fuel pyrolysis. Methyl substitution in the angular ring of benz(a)anthracene, however, tends to deactivate the molecule, although 4,5-dimethylbenz(a)anthracene may be an exception. Carcinogenic trimethyl- and tetramethylbenz(a)anthracenes are known, and their relative potencies are comparable to the parent 7,12-DMBA. In general, free radical synthesis of polycyclic hydrocarbons by pyrolysis does not favor alkyl side chain formation.

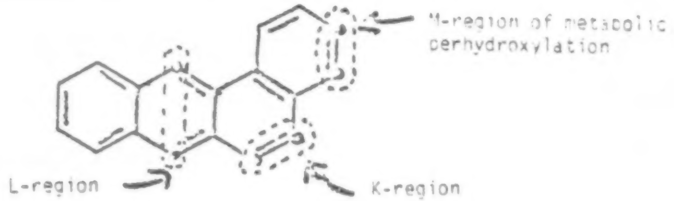
Alkyl substitution of partially aromatic condensed ring systems may also add considerable carcinogenic activity. The best example of this type of activation is 3-methylcholanthrene, a highly potent carcinogen.

With alkyl substituents longer than methyl, carcinogenicity tends to decrease, possibly due to a decrease in transport through cell membranes. However, different positions in the benz(a)anthracene molecule will vary with respect to the effect of n-alkyl substitution on carcinogenicity. Benz(a)anthracene is especially sensitive to decreased carcinogenicity caused by the addition of bulky substituents at the 7-position, and is indicative of a once widely-held view for most polycyclics that high reactivity of the meso-phenanthrenic region (now called the "K-region") was a critical determinant for carcinogenicity. Current studies show that the K-region is not involved in critical binding to DNA. The substitution of highly polar groups (e.g., -OH, -COOH) in the 7-position of benz(a)anthracene abolishes tumorigenic activity whereas a wide variety of less-polar substituents can enhance activity in position 7 (e.g., $-CH_2OH$, $-CH_2CH_2OH$, $-CH_2COONa$, $-CH_2COOCH_3$, $-CH_2OOCCH_3$, -CN, $-CH_2CN$, -CHO, $-NH_2$, -SH, $-COCCl_3$, $-OCH_3$).

Recent studies have indicated that methylation of the angular "bay region" (see Effects section) of the benzene ring, not only in benz(a)anthracene but also in other four, five, and six-ring aromatic hydrocarbons, leads to a significant decrease, or even to elimination, of the carcinogenic activity of the molecule. Methylation in other postions does not diminish, but frequently increases, carcinogenicity. For example, 7- and 8-methyl-BaP are inactive, whereas 2-,3-, 4-,5-,6-,11-, and 12-methyl-BaP are strong carcinogens.

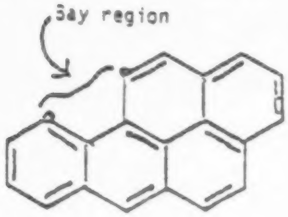
Partial hydrogenation of the polycyclic aromatic skeleton can generally be expected to decrease carcinogenic potency. This was shown with various hydrogenated derivatives of BaP, benz(a)anthracene, and MCA. On the other hand, the carcinogenicity of DBahA, dibenzo(a,i)pyrene, and dibenzo(a,h)-pyrene is not significantly altered by meso-hydrogenation. This may be due to the fact that extensive resonance capability is preserved. Moreover, 5,6-dihydro-DBahA actually displayed a fourfold increase in carcinogenicity in comparison to the parent hydrocarbon (Arcos and Argus, 1974), possibly due to the hydrophilicity and ease of intracellular transport of its dihydrodiol derivative.

For many years, investigators have sought a common molecular feature among PAH carcinogens which would serve to explain their biological activity. The "electronic theory of carcinogenesis" has relied upon an analysis of the influence of electron density at specific molecular regions to explain unique reactivity with cellular constituents. A basic assumption arising from the work of the Pullmans and other (Pullman and Pullman, 1955) was that a meso-phenanthrenic region ("K-region") of high π-electron density and with a propensity for addition reactions was a critical structural feature for polycyclic carcinogens. In expanding this hypothesis, further biological significance was attributed to the concomitant presence of a rather unreactive meso-anthracenic region ("L-region") for high carcinogenicity. In addition, a region of comparatively low reactivity which characteristically undergoes metabolic perhydroxylation (corresponding to the 3,4-positions of benz(a)anthracene) has been designated the M-region. According to the theory, only binding of the K-region to critical cellular sites would cause tumor formation; protein binding at the L-region causes no tumorigenic effect, while inactivation is produced by metabolic perhydroxylation in the M-region. The three regions of reactivity are readily distinguished in the benz(a)antrhacene skeleton:

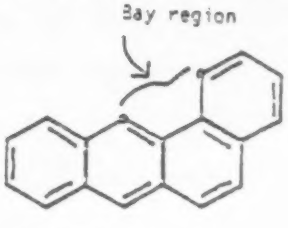


The electronic K-L theory of carcinogenic reactivity has encountered numerous inconsistencies, primarily because these relationships were derived from physical-chemical properties of the parent hydrocarbon and gave no consideration to the biological effects of activated metabolites.

Advances in recent years have focused attention on the potential reactivity of diol epoxide metabolites of PAH, and their ease of conversion to triol carbonium ions. Under the assumption that diol epoxides, which are more readily converted to carbonium ions, will be better alkylating agents to produce carcinogenesis and mutagenesis, the "bay region" theory has been proposed (Lehr, et al. 1978; Wood, et al. 1977). Examples of a "bay region" in a polycyclic hydrocarbon are the regions between the 10 and 11 positions of BaP and the 1 and 12 positions of benz(a)anthracene:



Benzo[a]pyrene



Benz[a]anthracene

The theory predicts that diol epoxides in which the oxirane oxygen forms part of a "bay region" (e.g., BaP 7,8-diol-9,10-epoxide) will be more reactive and hence more carcinogenic than diol epoxides in which the oxirane oxygen is not situated in a "bay region." Experimentally, the "bay region" diol epoxides of benz(a)anthracene, BaP, and chrysene were more mutagenic *in vitro* and/or tumorigenic than other diol epoxide metabolites, their precursor dihydrodiols, the parent hydrocarbons, or other oxidative metabolites. Moreover, quantum mechanical calculations were in accord with the concept that reactivity at the "bay region" is highest for all the diol epoxides derived from polycyclic hydrocarbons.

The bay region concept has received enough confirmation to lead to suggestions that an analysis of theoretical reactivity in this manner may be useful in screening PAH as potential carcinogens (Smith, et al. 1978). Among several indices of theoretical reactivity examined, the presence of a bay region for a series of PAH displayed a high degree of correlation with positive carcinogenic activity (Table 20).

TABLE 20

Reactivity Indicies for Polycyclic Hydrocarbons (Smith, et al. 1978)

Compound	K-region?	L-region?	Bay region	Carcinogenicity index Arcos and Argus (1974)	Carcinogenicity index Jerina, et al. (1977)
Naphthalene	–	–	–	0	–
Anthracene	–	+	–	0	–
Tetracene	–	+	–	0	–
Pentacene	–	+	–	0	–
Hexacene	–	+	–		?
BA	+	+	+	5	+
Benzo(a)tetracene	+	+	+		–
Phenanthrene	+	–	+	0	–
Benzo(c)phenanthrene	+	–	+	4	+
Chrysene	+	–	+	3	+
Benzo(b)chrysene	+	+	+		–
Picene	+	–	+	0	–
Triphenylene	–	–	+	0	–
Benzo(g)chrysene	+	–	+	17	++
Dibenz(a,c)anthracene	–	+	+	3	+
Dibenz(a,j)anthracene	+	+	+	4	+
Dibenz(a,h)anthracene	+	+	+	26	++
Naphtho(2,3-b)pyrene	+	+	a	[illegible]	++
Benzo(a)pyrene	+	–	+	73	++++
Benzo(e)pyrene	+	–	+	2	+
Dibenzo(a,l)pyrene	+	–	+	33	++[b]
Dibenzo(a,i)pyrene	+	–	+	74	++++
Dibenzo(a,e)pyrene	+	–	+	50	+++
Dibenzo(a,h)pyrene	+	–	+	70	++++
Tribenzo(a,e,i)pyrene	–	–	+	16	++

[a] This compound does not strictly possess a bay region but does contain a "pseudo" bay region.

[b] Jerina, et al. (1977) have assigned this as ++++.

The carcinogenic activity of BaP has been studied extensively in various animal model systems. In recent years, research on BaP has been expanded to include an examination of the tumorigenic activity of various BaP metabolites. These efforts were directed at the objective of identifying a BaP derivative which acts as the principal ultimate carcinogen resulting from metabolic activation (Levin, et al. 1977, 1976a,b; Slaga, et al. 1977, 1976; Kapitulnik, et al. 1976a,b; Wislocki, et al. 1977; Conney, et al. 1977a,b.

Studies on the activity of BaP and its derivatives as complete carcinogens on mouse skin (Table 21) and as tumor initiators (Table 22) revealed that marked differences in tumorigenic potency exist. The apparent lack of activity for the BaP 7,8-diol-9,10-epoxides, despite their exceptional mutagenicity, may be due to poor skin penetration of adult mouse skin because of high chemical reactivity. Indeed, as a carcinogen in newborn mice the (-) enantiomer of BaP, 7,8-dihydrodiol, and the 7,8-diol-9,10-epoxide derived therefrom are far more active than the parent hydrocarbon (Kapitulnik, et al. 1977, 1978a,b). These studies on the newborn mouse clearly indicate the role of a BaP 7,8-diol-9,10-epoxide as an ultimate carcinogenic metabolite of BaP.

Further dose-response information on the sarcomagenic activity of BaP by subcutaneous injection to rats and mice is summarized in Table 23.

Temporal relationships for the development of BaP-induced skin cancers in mice have been examined by Albert, et al. (1978). Their results showed that increasing weekly doses of BaP caused a shortening of the latency period for carcinoma

TABLE 21

Skin Tumors in Mice Treated with Benzo(a)pyrene and Derivatives

Treatment[a]	Total no. animals	Dose, µmoles	Mice with tumors, %	Total no. skin tumors[b]	Reference
BaP	25	0.4	100	32	Wislocki, et al. 1977
BaP	30	0.4	100	34	Wislocki, et al. 1977
BaP	26	0.4	92	34	Albert, et al. 1978
BaP	30	0.15	100	40	Levin, et al. 1976
BaP	27	0.1	96	28	Wislocki, et al. 1977
BaP	30	0.1	38	13	Levin, et al. 1977a
BaP	30	0.1	50	15	Levin, et al. 1977a
BaP	30	0.1	91	24	Levin, et al. 1977a
BaP	30	0.05	59	20	Levin, et al. 1977a
BaP	30	0.025	7	2	Levin, et al. 1977a
BaP	30	0.02	4	1	Levin, et al. 1977a
BaP	30	0.02	0	0	Levin, et al. 1977a
1-HOBaP	25	0.4	0	0	Wislocki, et al. 1977
2-HOBaP	29	0.4	100	37	Wislocki, et al. 1977
3-HOBaP	29	0.4	0	0	Wislocki, et al. 1977
4-HOBaP[c]	26	0.4	0	0	Albert, et al. 1978
5-HOBaP[c]	26	0.4	0	0	Albert, et al. 1978
6-HOBaP[c]	28	0.4	0	0	Albert, et al. 1978
7-HOBaP[c]	30	0.4	0	0	Albert, et al. 1978
8-HOBaP[c]	27	0.4	0	0	Albert, et al. 1978
9-HOBaP[c]	26	0.4	0	0	Albert, et al. 1978
10-HOBaP[c]	28	0.4	0	0	Albert, et al. 1978
11-HOBaP	28	0.4	14	4	Wislocki, et al. 1977
12-HOBaP	23	0.4	0	0	Wislocki, et al. 1977

[a] Female C57BL/6J mice were treated with BaP or BaP derivatives (0.02-0.4 µmole) once every 2 weeks for 60 weeks by topical application to the shaved skin of the back.

[b] Skin tumors consisted mostly of squamous cell carcinomas; other skin tumors were fibrosarcomas, papillomas, and keratocanthomas.

[c] Mice were treated once every 2 weeks for 56 weeks.

TABLE 21 (cont'd)

Skin Tumors in Mice Treated with Benzo(a)pyrene and Derivatives

Treatment[a]	Total no. animals	Dose, µmoles	Mice with tumors, %	Total no. skin tumors[b]	Reference
BaP 4,5-oxide	30-39	0.4	4	1	Levin, et al. 1976a
BaP 4,5-oxide	30-39	0.1	6	2	Levin, et al. 1976a
BaP 7,8-oxide	30-39	0.4	94	37	Levin, et al. 1976a
BaP 7,8-oxide	30	0.3	53	16	Levin, et al. 1976a
BaP 7,8-oxide	30	0.15	18	5	Levin, et al. 1976a
BaP 7,8-oxide	30-39	0.1	9	3	Levin, et al. 1976a
BaP 9,10-oxide	30-39	0.4	0	0	Levin, et al. 1976a
BaP 11,12-oxide	28	0.4	0	0	Wislocki, et al. 1977
BaP 11,12-oxide	17	0.1	0	0	Wislocki, et al. 1977
BaP 7,8-dihydrodiol	30	0.3	100	42	Levin, et al. 1976b
BaP 7,8-dihydrodiol	30	0.15	100	40	Levin, et al. 1976b
BaP 7,8-dihydrodiol	30	0.1	92	28	Levin, et al. 1976a
BaP 7,8-dihydrodiol	30	0.05	76	24	Levin, et al. 1976a
BaP 7,8-dihydrodiol	30	0.025	7	2	Levin, et al. 1976a
(±)-7β,8α-Dihydroxy-9β,10β-eopxy-7,8,9,10-tetrahydrobenzo(a)pyrene (diol epoxide 1)	30	0.4	0	0	Levin, et al. 1976a
diol epoxide 1	30	0.1	0	0	Levin, et al. 1976a
diol epoxide 1	30	0.02	0	0	Levin, et al. 1976a

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[a] Female C57BL/6J mice were treated with BaP or BaP derivatives (0.02-0.4 µmole) once every 2 weeks for 60 weeks by topical application to the shaved skin of the back

[b] Skin tumors consisted mostly of squamous cell carcinomas; other skin tumors were fibrosarcomas, papillomas, and keratocanthomas.

TABLE 21 (cont'd)

Skin Tumors in Mice Treated with Benzo(a)pyrene and Derivatives

Treatment[a]	Total no. animals	Dose, µmoles	Mice with tumors, %	Total no. skin tumors[b]	Reference
(±)-7β,8α-Dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene (diol epoxide 2)	30	0.4	13	3	Levin, et al. 1976a
diol epoxide 2	30	0.1	7	2	Levin, et al. 1976a
diol epoxide 2	30	0.02	0	0	Levin, et al. 1976a

[a] Female C57BL/6J mice were treated with BaP or BaP derivatives (0.02-0.4 µmole) once every 2 weeks for 60 weeks by topical application to the shaved skin of the back.

[b] Skin tumors consisted mostly of squamous cell carcinoas; other skin tumors were fibrosarcomas, papillomas, and keratocathomas.

TABLE 22

Summary of the Skin Tumor Initiation Activities of Benzo(a)pyrene and its Metabolites

Initiator	No. mice	Dose, hmoles	Weeks of promotion	Mice with tumors, %	Papillomas/ mouse	Reference
BaP	30	200	23	94	4.8	Slaga, et al. 1976
BaP	30	200	30	92	5.3	Slaga, et al. 1977
BaP	30	200	21	77	2.6	Levin, et al. 1977b
BaP 4,5-epoxide	30	200	23	20	0.2	Slaga, et al. 1976
BaP 7,8-epoxide	29	200	23	81	1.9	Slaga, et al. 1976
BaP 9,10-epoxide	29	200	30	15	0.15	Slaga, et al. 1977
BaP 11,12-epoxide	30	200	30	38	0.45	Slaga, et al. 1977
BaP 7β,8α-diol-9α,10α-epoxide	29	200	30	69	1.5	Slaga, et al. 1977
BaP 7β,8α-diol-9β,10β-epoxide	28	200	30	7	0.07	Slaga, et al. 1977
BaP 7,8-dihydrodiol	29	200	30	86	5.0	Slaga, et al. 1977
(-)-BaP 7,8-dihydrodiol[b]	30	100	21	77	3.8	Levin, et al. 1977b
(+)-BaP 7,8-dihydrodiol[b]	30	100	21	23	0.43	Levin, et al. 1977b

[a] Female CD-1 mice were treated with a single dose of initiator dissolved in acetone, acetone: NH_4OH (1000;1), or dimethyl sulfoxide:acetone (1;3) and followed 1 week later by twice-weekly applications of 10 μg of TPA.

[b] Promotion was by twice-weekly applications of 16 hmoles of TPA beginning 11 days after treatment with initiator.

TABLE 23

Induction of Sarcoma by Benzo(a)pyrene

Species	No. and (sex)	Total dose µmoles	Animals with sarcoma, %	Average latency, days	Reference
Rat (Sprague-Dawley)	13 (female)	6.0[a]	100	101 ± 2.7	Flesher, et al. 1976
Mouse	14 (male)	7.1[b]	93	129	Buu-Hoi, 1964
Mouse	16 (female)	7.1[b]	50	160	Buu-Hoi, 1964
Mouse	9 (?)	15.9[c]	66.6	112	Gottschalk, 1942
Mouse	10 (?)	5.0[c]	70	122	Gottschalk, 1942
Mouse	12 (?)	0.5[c]	66.6	155	Gottschalk, 1942
Mouse	15 (?)	0.002[c]	0	N.A.[d]	Gottschalk, 1942

[a] Administered as 0.2 µmole dissolved in 0.1 ml sesame oil by subcutaneous injection on alternate days for 30 doses beginning at 30 days of age.

[b] Administered as three injections of 2.4 µmoles each, given at 1 month intervals.

[c] Administered as a single injection under the skin of the abdomen, dissolved in 0.5 ml of neutral olive oil.

[d] Not applicable.

formation. Furthermore, it was determined that the development of papillomas as a precursor lesion to carcinoma formation occurred only at higher BaP doses (e.g., 32 µg and 64 µg per week). At the lower dose levels (8 µg and 16 µg per week), carcinomas appeared de novo without precursor papilloma formation.

The carcinogenicity of BaP by oral intake has not been studied as throughly as for other routes of administration. Nevertheless, tumors of various sites result when BaP is administered orally to rodents (Table 24).

With oral, intratracheal, and intravenous routes of administration, BaP is less effective than other PAH (e.,g., DMBA, MCA, dibenz(a,h)anthracene) in producing carcinomas. On the other hand, BaP has remarkable potency for the induction of skin tumors in mice. Therefore, caution must be exercised in considering the carcinogenicity of PAH as a class, and in extrapolating data derived from studies with BaP to the effects of PAH mixtures.

An examination of comparative carcinogenicities within the same tumor model system can provide valuable insight concerning relative risks of various PAH. By single intravenous injection of about 0.25 mg of aqueous dispersions of PAH to mice, a direct comparison of carcinogenic potency was possible (Table 25). In this test system, MCA displayed the greatest lung tumor-forming capability; dibenz(a,h)anthracene followed closely in activity with BaP being considerably less potent.

TABLE 24

Carcinogenicity of Benzo(a)pyrene by Oral Administration to Various Mammals (IARC, 1973)

Compound	Species	Dose	Route of administration	Effects
BaP	Mouse	0.2 mg in PEG[a]	Intragastric	14 tumors of the forestomach in 5 animals out of 11
	Mouse (age 17-116 days)	50-250 ppm	Dietary (110-197 days)	79% incidence of stomach tumors at 50-250 ppm for 197 days; no tumors with diets containing up to 30 ppm for 110 days
	Mouse	250 ppm	Dietary	100% stomach tumor incidence when diet was fed for 30 days; 5-7 days of feeding, 30-40%; 2 to 4 days of feeding, 10 percent; 1 day of feeding, 0 percent
	Mouse (age 18-30 days)	250 ppm	Dietary (140 days)	Leukemias, lung adenomas, and stomach tumors produced
	Rat (Sprague-Dawley; age 105 days)	2.5 mg per day	Oral	Papillomas developed in the esophagus and forestomach in 3 out of 40 animals
	Hamster	2-5 mg bi-weekly	Intragastric	5 stomach papillomas in 67 animals treated for 1-5 months; 7 papillomas and 2 carcinomas in 18 animals treated for 6-9 months; 5 papillomas in 8 animals treated for 10-11 months
	Hamster	500 ppm	Dietary (4 days per week for up to 14 months)	12 tumors (2 esophagus, 8 forestomach, 2 intestinal) in 8 animals

[a] Polyethylene glycol

TABLE 25

Comparative Carcinogenicity of Polycylic Hydrocarbons and Related Compounds Measured by Induction of Lung Tumors (LT) (Shimkin and Stoner, 1975)[a]

Compound	Dose, µmoles/kg	Mice with LT/ no. of mice	Mean no. LT/mouse	µMoles/kg for 1 LT response
3-Methylcholanthrene, 0.1 mg	15	15/15	11	0.9
3-Methylcholanthrene, 0.5 mg	74	6/6	47	
Dibenz(a,h)anthracene	36	10/10	31	1.0
7H-Dibenzo(c,g)carbazole	38	12/12	5.7	6.0
Benzo(a)pyrene	40	10/10	3.7	9.5
Dibenz(a,j)aceanthrylene	33	9/10	2.7	14
Dibenz(a,h)acridine	36	11/12	2.0	18
8-Methylbenzo(c)phenanthrene	42	6/11	0.7	--
7-Methylbenzo(a)pyrene	38	5/10	0.6	--
5-Methoxy-7-propylbenz(a)anthracene	33	1/10	0.1	--
Benz(a)anthracene	44	2/11	0.2	--
Untreated controls	--	4/19	0.2	--

[a] Strain A mice, 8-12 weeks old, received single intravenous injection of 0.24 mg of methlcholanthrene in aqueous dispersion and were killed 20 weeks later.

Intratracheal instillation of PAH to Syrian golden hamsters has been widely utilized for the conduct of studies on pulmonary carcinogenesis (Saffiotti, et al. 1968, 1972; Henry, et al. 1975). Several studies are summarized in Table 26 and indicate that: (1) dose-response relationships are clearly evident, and (2) the co-administration of carrier particles such as Fe_2O_3 (i.e., with BaP) can markedly increase tumor incidence, depending on the conditions of the experiment and physical characteristics of the particle. Since environmental exposures to PAH occur in conjunction with particulate material in air, this effect may be particularly relevant to human situation.

In addition to the hamster model system, respiratory tract tumors have been readily induced by PAH in rats and mice. The results of several representative studies are summarized in Table 27.

The published literature regarding chemical carcinogenesis in cell cultures is vast, despite the fact that systematic studies were not begun until the early 1960's due to the lack of a reproducible transformation assay. Berwald and Sachs (1963) first demonstrated that polycyclic hydrocarbons (MCA, BaP) could cause the direct malignant transformation of hamster embryo cells in culture. Transformed colonies have growth characteristics visually distinct from normal colonies and are readily seen above a background of normal cells. This assay can therefore be easily used as a screen to compare carcinogenic activity of suspect compounds. A common feature of these, and nearly all, trans-

TABLE 26

Induction of Respiratory Tract Tumors in Syrian Golden Hamsters by Intratracheal Instillation of PAH

Compound	No. animals	Total dose, mg	Respiratory tumor incidence, percent	Reference
BaP	30	3.25^a	10	Feron, et al. 1973
BaP	30	6.5^a	13	Feron, et al. 1973
BaP	30	13	30	Feron, et al. 1973
BaP	29	26^a	86	Feron, et al. 1973
BaP	28	52^a	93	Feron, et al. 1973
BaP	48	30^b	15	Sellakumar, et al. 1976
BaP and Fe_2O_3	48	30^b	71	Sellakumar, et al. 1976
BaP and Fe_2O_3, coated	49	26.1^c	73	Henry, et al. 1975
BaP and Fe_2O_3, ground	49	27.4^c	84	Henry, et al. 1975
BaP and Fe_2O_3, mixed	43	26.3^c	12	Henry, et al. 1975
BaP and gelatin	46	26.4^c	17	Henry, et al. 1975
BaP and Fe_2O_3	28 (male), 29 (female)	60^d	60.7 (male), 58.6 (female)	Saffioti, et al. 1972
BaP and Fe_2O_3	33 (male), 34 (female)	30^d	66.7 (male), 58.8 (female)	Saffioti, et al. 1972
BaP and Fe_2O_3	33 (male), 30 (female)	15^d	30.3 (male), 30.0 (female)	Saffioti, et al. 1972
BaP and Fe_2O_3	47 (male), 41 (female)	7.5^d	12.8 (male), 9.8 (female)	Saffioti, et al. 1972
BaP	32 (male)	30^e	42.3	Kobayashi, 1975
BaP	28 (female)	30^e	57.7	Kobayashi, 1975
DB(a,i)P	48	12^f	75	Stenback and Sellakumar, 1974a
DB(a,i)P	48	8.5^g	64.6	Stenback and Sellakumar, 1974a
DMBA and Fe_2O_3	46	1.2^h	43.5	Stenback and Sellakumar, 1974b
DMBA and Fe_2O_3	28	0.85	46.4	Stenback and Sellakumar, 1974b

[a] Animals treated once weekly for 52 weeks with BaP suspended in 0.9% NaCl solution.

[b] 3 mg BaP administered once weekly for 10 weeks.

[c] Animals received 30 weekly intratracheal instillations.

[d] Animals received 30 weekly instillations of BaP mixed with equal amounts of Fe_2O_3 and suspended in 0.2 ml saline.

[e] Animals received 30 weekly intratracheal instillations of BaP suspended in 0.9% NaCl.

[f] Animals received 12 weekly intratracheal instillations of 1 mg DB(a,i)P suspended in distilled water.

[g] Animals received 17 weekly intratracheal instillations of 0.5 mg DB(a,i)P suspended in distilled water.

[h] Animals received 100 µg DMBA and 100 µg Fe_2O_3 intratracheally once a week for 12 weeks in saline suspensions.

[i] Animals received 50 µg DMBA and 50 µg Fe_2O_3 intratracheally once a week for 17 weeks in saline suspensions.

TABLE 27

Induction of Respiratory Tract Tumors in Rats and Mice

Compound	Organism	No. animals	Total dose, mg	Route of administration	Tumor incidence, %	Reference
DMBA and Indian ink	Rat (Wistar and random-bred)	34	2.5[a]	Intratracheal instillation	17.6	Pylev, 1962
DMBA and Indian ink	Rat (Wistar and random-bred)	56	6[b]	Intratracheal instillation	35.7	Pylev, 1962
DMBA and Indian ink	Rat (Wistar and random-bred)	61	10[c]	Intratracheal instillation	26.2	Pylev, 1962
DB(a,h)A	Mouse (DBA/2)	14 (male) 13(female)	236(male)[d] 179 (female)[d]	Oral	100 (male)[e] 77 (female)[e]	Snell and Stewart, 1962
MCA	Rat (Osborne-Mendel)	100	0.005[f]	Pulmonary injection	1[g]	Hirano, et al. 1974
MCA	Rat (Osborne-Mendel)	100	0.05[f]	Pulmonary injection	13[g]	Hirano, et al. 1974
MCA	Rat (Osborne-Mendel)	100	0.10[f]	Pulmonary injection	27[g]	Hirano, et al. 1974

TABLE 27 (contd)

Induction of Respiratory Tract Tumors in Rats and Mice

Compound	Organism	No. animals	Total dose, mg	Route of administration	Tumor incidence, %	Reference
MCA	Rat (Osborne-Mendel)	100	0.20[f]	Pulmonary injection	47[g]	Hirano, et al. 1974
MCA	Rat (Osborne-Mendel)	100	0.30[f]	Pulmonary injection	40[g]	Hirano, et al. 1974
MCA	Rat (Osborne-Mendel)	100	0.40[f]	Pulmonary injection	51[g]	Hirano, et al. 1974
MCA	Rat (Osborne-Mendel)	100	0.50[f]	Pulmonary injection	45[g]	Hirano, et al. 1974

[a] Administered as a single dose with 0.2 mg of Indian ink in 0.2 ml of a colloid protein solution.
[b] Administered as three 2 mg doses at monthly intervals with 0.2 mg of Indian ink in 0.2 ml of a colloid protein solution.
[c] Administered as five 2 mg doses at monthly intervals with 0.2 mg of Indian ink in 0.2 ml of a colloid protein solution.
[d] Administered as an aqueous-olive oil emulsion of DB(a,h)A given in place of drinking water for 237 to 279 days.
[e] Tumors were alveologenic carcinomas, a 100% incidence of pulmonary adenomatosis was also observed.
[f] Administered as a single MCA-containing beeswax pellet placed directly into the lower peripheral segment of the left lung.
[g] Overt squamous cell carcinoma.

formed cells is that they give rise to fibrosarcomas upon inoculation into immunosuppressed animals. In addition to hamster embryo cells, malignant transformation has been demonstrated in organ cultures, liver cell cultures, fibroblastic cells derived from mouse ventral prostate, 3TC cell lines derived from mouse embryo cells, and various types of epithelial cells from humans and other animals (Heidelberger, 1973, 1975a,b).

Early reports by Berwald and Sachs (1965) and Dipaolo and Donovan (1967) described alterations in hamster embryo cells induced by BaP, DMBA, and MCA which could be used as indicators of a change from normal to neoplastic state. The compounds were applied to cells in culture either dissolved in paraffin and impregnated on filter disks or as a colloidal suspension in growth medium. Following marked cytotoxicity, foci of transformed cells developed which displayed continuous proliferation *in vitro*, chromosomal abnormalities, and the ability to grow indefinitely in culture. In addition, these transformed mass cultures, when transplanted to four- to six-week old hamsters, continued to grow and form tumors. A good correlation was obtained between *in vitro* carcinogenicity of a polycyclic hydrocarbon and the number of transformed clones they produced. The maximum rate of cell transformation in these studies was 25.6 percent in surviving cells, obtained by treatment with 10 µg/ml of BaP for six days. BaP treatment at 1 µg/ml for six days produced 19.9 percent transformation in surviving cells. Further data indicating the activity of several polycyclic carcinogens and their derivatives are summarized in Table 28. The K-region epoxides of DBahA and MCA are more active in the production of malignant transformation in hamster embryo cells than the parent hydrocarbons or the corresponding K-region phenols (Grover, et al. 1971; Huberman, et al. 1972). Although these results confirm the view that metabolism is necessary for carcinogenic activity, they conflict with data generated *in vivo* (see Effects section) which indicate that K-region epoxides of polycyclic carcinogens are less active than the parent compound in various species. A possible reason for the lack of correlation is the relative instability of K-region epoxides as compared to the parent hydrocarbon when applied to the skin. It is likely that *in vivo* far less of the reactive K-region epoxide can survive passage through the skin to reach the basal cell layer. Furthermore, it has become apparent that the non-K-region diol-epoxide is likely to be the ultimate carcinogenic metabolite for most PAH. Several investigators have also made it evident that the toxicity and transforming activity of PAH are dissociable and occur by different processes (Landolph, et al. 1976; DiPaolo, et al. 1971), with the toxicity being due to random alkylation of nucleophilic regions within the cell. However, when hamster embryo cells are pretreated with weak chemical carcinogens which can induce microsomal enzyme activity (e.g., benz(a)anthracene, methyl methanesulfonate, ethyl methanesulfonate) before the addition of a potent carcinogen (e.g., MCA, BaP, DMBA), transformation may be considerably enhanced (DiPaolo, et al. 1971, 1974).

As a prescreen for chemical carcinogens, cell transformation *in vitro* may be one of the most sensitive techniques available. Pienta and coworkers (1977) reported that 90 percent (54/60) of the carcinogens they tested transformed hamster embryo cells *in vitro*, whereas none of the noncarcinogens tested showed any activity. Moreover, many of the carcinogens which have not been shown to be mutagenic toward *S. tymphimurium in vitro* (e.g., chrysene) were capable of transforming the hamster cells. It is noteworthy, however, that large differences exist in dosage requirements for transformation among those various test systems. Calculations have been made which show that a battery of tests using *S. typhimurium* (Ames assay), polymerase A-deficient *E. coli*, and hamster embryo cell transformation is capable of detecting nearly all carcinogens tested, both PAH and non-PAH types.

The alteration of microsomal enzyme activity either *in vitro* or *in vivo* is known to have a marked effect on the carcinogenic response to PAH. Nesnow and Heidelberger (1976) reported that in 10T1/2CL8 cells, a line of contact-sensitive C3H mouse embryo fibroblasts, transformation in culture was altered by chemical modifiers of microsomal enzymes. Pretreatment of 10T1/2C18 cells with benz(a)anthracene, a microsomal enzyme inducer, caused a doubling in MCA-mediated transformation. Similarly, treatment with inhibitors of epoxide hydrase (e.g., cyclohexene oxide;

TABLE 28

Hamster Embryo Cell Transformation Produced by Several Polycyclic Hydrocarbons and Their Derivatives

Compound	Concentration, ug/ml	Total no. colonies	Cloning efficiency, %	No. transformed colonies	Transformation, %	Reference
DB(a,h)A[a]	2.5	760	4.2	4	0.5	Huberman, et al. 1972
	5	690	3.8	4	0.7	Huberman, et al. 1972
	10	790	4.4	7	0.9	Huberman, et al. 1972
DB(a,h)A[b]	2.5	1341	13.4	3	0.2	Grover, et al. 1971
	5.0	1363	14.0	11	0.8	Grover, et al. 1971
	10	1365	14.5	7	0.5	Grover, et al. 1971
DB(a,h)A5,6-epoxide[a]	2.5	598	3.3	3	0.5	Huberman, et al. 1972
	5	601	3.3	12	2.0	Huberman, et al. 1972
	7.5	395	2.5	31	7.8	Huberman, et al. 1972
	10	350	1.9	14	4.0	Huberman, et al. 1972
DB(a,h)A5,6-epoxide[b]	2.5	895	10.1	7	0.8	Grover, et al. 1971
	5.0	866	9.3	20	2.3	Grover, et al. 1971
	7.5	817	9.3	22	2.7	Grover, et al. 1971
	10	707	7.7	30	4.2	Grover, et al. 1971
MCA[c]	2.5	404	10.1	9	2.2	Huberman, et al. 1972
	5	370	9.2	10	2.7	Huberman, et al. 1972
	7.5	349	8.7	15	4.3	Huberman, et al. 1972
MCA[d]	2.5	664	9.6	20	3.46	DiPaolo, et al. 1971
MCA epoxide[c]	3.5	364	2.4	13	3.6	Huberman, et al. 1972
	5	245	1.5	8	3.3	Huberman, et al. 1972
	7	103	0.7	17	16.5	huberman, et al. 1972
BaP[d]	1	1016	8.46	25	2.46	DiPaolo, et al. 1971
	5	394	7.17	21	5.33	DiPaolo, et al. 1971

[a] 7-day treatment of cells seeded on a feeder layer.
[b] 7-8 day treatment of cells.
[c] 4-hour treatment of cells seeded in conditioned medium.
[d] 8-day treatment of cells.

styrene oxide; 1,2,3,4-tetra-hydronaphthalene-1,2-oxide)caused an increase in transformation over that obtained with MCA treatment alone. Thus, treatments which can induce epoxide-forming enzymes and/or lower the activity of epoxide-degrading enzymes seemed to enhance the degree of transformation in cultured cells by altering steady-state levels of oncogenic epoxides.

Chen and Heidelberger (1969a,b) developed a system using C3H mouse ventral prostate cells to examine transformation by carcinogenic hydrocarbons under conditions in which no spontaneous malignant transformation occurred. Cells treated with MCA (1 µg/ml) for six days in culture produced malignant fibrosarcomas in 100 percent of mice into which they were subcutaneously injected. When treated for only one day with MCA at the single cell stage, transformed foci were found in all clones grown to confluency. A good quantitative correlation was obtained between the *in vivo* oncogenic activity of eight hydrocarbons (including BaP, MCA, DMBA, and DBahA) and the number of transformed colonies produced in this system. In contrast to the enhanced transforming ability of K-region epoxides relative to the parent hydrocarbon in hamster embryo cells, the K-region epoxide derived from DMBA was less active and the K-region epoxides from MCA, DBahA, and benz(a)anthracene were more active than the parent compound in mouse prostrate cells (Marquardt, et al. 1972, 1974). Moreover, the epoxide derived from DMBA was more toxic than DMBA itself. The anomalous behavior of DMBA may have been due, however, to a decreased intracellular half-life of the epoxide because of its greater chemical reactivity.

Attempts to transform human cells in culture with PAH (e.g., BaP, MCA, DMBA) have generally met with failure (Leith and Hayflick, 1974). However, Rhim and coworkers (1975) reported that a human osteosarcoma clonal cell line could be further transformed *in vitro* with DMBA. Morphologic alterations and abnormal growth patterns became evident in cells treated with DMBA at 2.5 and 1.0 µg/ml in the fifth subculture 52 to 57 days after exposure. One of the altered cell lines obtained from the 1 µg/ml treatment was tumorigenic in nude mice by subcutaneous and intracerebral injection. Interpretation of the significance of these results is made difficult by the fact that an aneuploid sarcomatous cell line had to be employed in order to demonstrate successful transformation.

The use of organ cultures for the assessment of chemical carcinogenicity suffers from the lack of reliable biochemical and morphological parameters for measuring early neoplastic changes. Nevertheless, pioneering work in the application of organ culture to chemical carcinogenesis was performed by Lasnitzki (1963). Microgram quantities of MCA added to organ cultures of rat and mouse prostate fragments caused extensive hyperplasia and squamous metaplasia. However, these preneoplastic morphological effects are generally not associated with subsequent tumor development when carcinogen-treated pieces of tissue are implanted into host animals (Heidelberger, 1973). Limited success has been achieved with organ cultures of rat tracheas, which showed characteristic morphologic alterations when treated with DMBA, BaP, and MCA (Heidelberger, 1973). In addition, Crocker (1970) has exposed respiratory epithelia from the hamster, rat, dog, and monkey to BaP at 7 to 15 ug/ml and observed occasional squamous metaplasia. More commonly, pleomorphic cells in a dysplastic epithelium were evident as a result of the treatment. Using this system, it was also possible to demonstrate a protective effect of vitamin A against BaP-induced abnormal differentiation. Rat tracheas maintained in organ culture have been suggested as a useful system for the predictive screening of potential carcinogens (Lindsay, et al. 1974).

A unique organ culture technique has recently been reported in which BaP (4 or 12 mg) was administered to pregnant mice (strain A and C57 Bl), and lung tissue of their 19- to 20-day-old embryos was subsequently explanted in culture (Shabad, et al. 1974). A transplacental influence of BaP was manifested as a proliferative stimulus in embryonic lung tissue. Hyperplasia arising in the bronchial epithelium led to the development of adenomas in a large percentage of the explants.

In the environment, man is unlikely to come in contact with only a single PAH, regardless of the route of exposure. Instead, PAH occur as complex mixtures in all environmental media. Despite this generally accepted fact, very few studies have been conducted on the carcinogenicity of defined PAH mixtures.

Among the most relevant studies conducted on the effects of PAH mixtures were those concerned with the carcinogenic components of automotive engine exhaust. Pfeiffer (1973,1977) treated groups of 100 female NMRI mice with single subcutaneous injections of a mixture containing 10 non-carcinogenic PAH, in addition to BaP and/or dibenz(a,h)anthracene. The treatment combinations and dosages are summarized in Table 29. As the results depicted in Table 30 indicate, increases in tumor incidence could be attributed to the presence of increased amounts of BaP and of dibenz(a,h)anthracene. It is noteworthy that, at the lower dosages, dibenz(a,h)anthracene was more effective in producing tumors at the injection site than was BaP. Moreover, no effect of the 10 non-carcinogens on tumorigenic response was evident. Probit analysis of tumor incidence data indicated that the tumorigenic response from application of all 12 PAH was attributable solely to dibenz(a,h)-anthracene.

Similar studies intended to reveal carcinogenic interactions among PAH found in automobile exhaust were conducted by Schmahl, et al. (1977). Eleven PAH were selected for their experiments, and various combinations were applied to the skin of NMRI mice in a proportion based on their respective weights in automobile exhaust (Table 31). Animals recieved twice weekly treatments for life (or until a carcinoma developed). Their results (Table 32) indicated that a mixture of carcinogenic PAH was more effective than BaP alone, and that the whole mixture (carcinogenic plus non-carcinogenic PAH) was not significantly more effective than the carcinogenic

TABLE 29

Classification of Test Groups
(Pfeiffer, 1977)

A	dose (ug)	substance	B	dose (ug)	substance
A_1	3.12	benzo(a)pyrene	B_1	2.35	dibenz(a,h)anthracene
A_2	6.25		B_2	4.7	
A_3	12.5		B_3	9.3	
A_4	25.0		B_4	18.7	
A_5	50.0		B_5	37.5	
A_6	100.0		B_6	75.0	

C

Substance	C_1 dose (µg)	C_2 dose (µg)	C_3 dose (µg)	C_4 dose (µg)	C_5 dose (µg)	C_6 dose (µg)
benzo(e)pyrene	2.15	4.3	8.75	17.5	35.5	70.0
benzo(a)anthracene	3.125	6.25	12.5	25.0	50.0	100.0
phenanthrene	125.0	250.0	500.0	1000.0	2000.0	4000.0
anthracene	31.25	62.5	125.0	250.0	500.0	1000.0
pyrene	65.1	131.2	262.5	525.0	1050.0	2100.0
fluoranthene	28.1	56.25	112.5	225.0	450.0	900.0
chrysene	3.125	6.25	12.5	25.0	50.0	100.0
perylene	0.2	0.4	0.87	1.75	3.5	7.0
benzo(ghi)perylene	12.8	25.6	51.25	102.5	205.0	410.0
coronene	3.125	6.25	12.5	25.0	50.0	100.0

D		E	
D_1	$A_1 + B_1$	E_1	$C_1 + D_1$
D_2	$A_2 + B_2$	E_2	$C_2 + D_2$
D_3	$A_3 + B_3$	E_3	$C_3 + D_3$
D_4	$A_4 + B_4$	E_4	$C_4 + D_4$
D_5	$A_5 + B_5$	E_5	$C_5 + D_5$
D_6	$A_6 + B_6$	E_6	$C_6 + D_6$

TABLE 30

Tumor Incidence Resulting, by the End of the 114th Week, from a Single Subcutaneous Application of Test Substances (Pfeiffer, 1977)

BAP group (A)		DBA group (B)		BaP + DAB group (D)	10 PAH group (C)	12 PAH group (E)
dose (ug)	no. of tumors	dose (ug)	no. of tumors	no. of tumors	no. of tumors	no. of tumors
3.12	9	2.35	37	48	6	41
6.25	35	4.7	39	44	8	55
12.5	51	9.3	44	61	6	61
25.0	57	18.7	56	68	4	72
50.0	77	37.5	65	69	13	68
100.0	83	75.0	69	79	5	82

TABLE 31

Doses (ug) Applied in Dermal Administration Experiments, in Relation to Benzo(a)pyrene (Schmahl, et al. 1977)

Controls				
Acetone	as solvent			
Benzo(a)pyrene	1.0	1.7	3.0	
C PAH				
Benzo(a)pyrene	1.0	1.7	3.0	
Dibenz(a,h)anthracene	0.7	1.2	2.1	
Benzo(a)anthracene	1.4	2.4	4.2	
Benzo(b)fluoranthene	0.9	1.5	2.7	
total	4.0	6.8	12.0	
NC PAH				
(Benzo(a)pyrene	1.0	3.0	9.0	27.0)
Phenanthrene	27.0	81.0	243.0	729.0
Anthracene	8.5	25.5	76.5	229.5
Fluoranthene	10.8	32.4	97.2	291.6
Pyrene	13.8	41.4	124.2	372.6
Chrysene	1.2	3.6	10.8	32.4
Benzo(e)pyrene	0.6	1.8	5.4	16.2
Benzo(ghi)perylene	3.1	9.3	27.9	83.7
total	65.0	195.0	585.0	1755.0
C PAH + NC PAH				
(Benzo(a)pyrene	1.0	1.7	3.0)	
Total C PAH	4.0	6.8	12.0	
Total NC PAH	65.0	110.5	195.0	
Total C PAH + NC PAH	69.0	117.3	207.0	

Relation of C PAH:NC PAH is constantly 1:16.25

TABLE 32

Findings at the Site of Application of PAH to Mouse Skin[a] (Schmahl, et al. 1977)

Application	Single dose ug	Initial no. of animals	Effective no. of animals	Histological diagnosis at the site of application							
				negative		papilloma		carcinoma		sarcoma	
				abs.	%	abs.	%	abs.	%	abs.	%
Solvent	-	100	81	80	99	-	-	-	-	1	1
BaP	1.0	100	77	66	86	1	1	10	13	-	-
BaP	1.7	100	88	63	72	-	-	25	28	-	-
BaP	3.0	100	81	36	44	2	3	43	53	-	-
C PAH	4.0	100	81	52	64	4	5	25	31	-	-
C PAH	6.8	100	88	31	35	3	3	53	60	1	1
C PAH	12.0	100	90	25	28	1	1	63	70	1	1
NC PAH	65.0	100	85	84	99	-	-	1	1	-	-
NC PAH	195.0	100	84	84	100	-	-	-	-	-	-
NC PAH	585.0	100	88	87	99	-	-	1	1	-	-
NC PAH	1755.0	100	86	70	81	-	-	15	17	1	1
C PAH + NC PAH	69.0	100	89	43	48	1	1	44	49	1	1
C PAH + NC PAH	117.3	100	93	36	39	2	2	54	58	1	1
C PAH + NC PAH	207.0	100	93	28	30	1	1	64	69	-	-

[a] The decimal points have been rounded off; therefore, the sum of % values will not always be equivalent to 100%

PAH group alone. Thus, the carcinogenic effects observed were solely attributable to the carcinogenic components of the misture.

Human data: Although exposure to PAH occurs predominantly by direct ingestion (i.e., in food and in drinking water) there are no studies to document the possible carcinogenic risk to humans by this route of exposure. It is known only that significant quantities of PAH can be ingested by humans, and that in animals such exposures are known to cause cancers at various sites in the body.

Convincing evidence from air pollution studies indicates an excess of lung cancer mortality among workers exposed to large amounts of PAH-containing materials such as coal gas, tars, soot, and coke-oven emissions (Kennaway, 1925; Kennaway and Kennaway, 1936, 1947; Henry, et al. 1931; Kuroda, 1937; Reid and Buck, 1956; Doll, 1952; Doll, et al. 1965, 1972; Redmond, et al. 1972, 1976; Mazumdar, et al. 1975; Hammond, et al. 1976; Kawai, et al. 1967). However, no definite proof exists that the PAH present in these materials are responsible for the cancers observed. Nevertheless, our understanding of the characteristics of PAH-induced tumors in animals, and their close resemblance to human carcinomas of the same target organs, strongly suggests that PAH pose a carcinogenic threat to man, regardless of the route of exposure.

The magnitude of the carcinogenic risk of PAH to man remains obscure in the community setting. Ambient levels of PAH in air are much lower than are encountered in occupational situations, and populations exposed are much more heterogeneous with regard to age, sex, and health status. However, the current state of knowledge regarding chemical carcinogenesis would lead to the conclusion that the number of cancers produced is directly proportional to the dose received by any route. One must assume, therefore, that the small amounts of PAH present in the environment (air, food, and water) under ambient conditions contribute in some degree to the observed incidence of lung cancer in most populations.

CRITERION FORMULATION

Existing Guidelines and Standards

There have been few attempts to develop exposure standards for PAHs, either individually or as a class. In the occupational setting, a Federal standard has been promulgated for coke oven emissions, based primarily on the presumed effects of the carcinogenic PAH contained in the mixture as measured by the benzene soluble fraction of total particulate matter. Similarly, the American Conference of Governmental Industrial Hygienists recommends a workplace exposure limit for coal tar pitch volatiles, based on the benzene-soluble fraction containing carcinogenic PAH. The National Institute for Occupational Safety and Health has also recommended a workplace standard for coal tar products (coal tar, creosote, and coal tar pitch), based on measurements of the cyclohexane extractable fraction. These standards are summarized below:

Substance	Exposure Limit	Agency
Coke Oven Emissions	150 $\mu g/m^3$, 8-hr. time-weighted average	U.S. Occupational Safety and Health Administration
Coal Tar Products	0.1 mg/m^3, 10-hr. time-weighted average	U.S. National Institute for Occupational Safety and Health
Coal Tar Pitch of Volatiles	0.2 mg/m^3 (benzene soluble fraction) 8-hr. time-weighted average	American Conference of Governmental Industrial Hygienists

A drinking water standard for PAH as a class has been developed. The 1970 World Health Organization European Standards for Drinking Water recommends a concentration of PAH not to exceed 0.2 µg/l. This recommended standard is based on the composite analysis of six PAH in drinking water: 1) fluoranthene, (2) benzo(a)pyrene, (3)benzo(g,h,i) perylene, (4) benzo(b)fluoranthene, (5) benzo(k)fluoranthene, and (6) indeno(1,3,-cd)pyrene.

The designation of these six PAH for analytical monitoring of drinking water was not made on the basis of potential health effects or bioassay data on these compounds (Borneff and Kunte, 1969). Thus, it should not be assumed that these six compounds have special significance in determining the likelihood of adverse health effects resulting from absorption of any particular PAH. They are, instead, considered to be useful [illegible]cators for the presence of PAH pollutants. Borneff and Kunte (1969) found that PAH were present in ground water at concentrations up to 50 ng/l, and in drinking water at concentrations up to 100 ng/l. Based on these data they suggested that water containing more than 200 ng/l should be rejected. However, as data from a number of U.S. cities indicate (see Exposure section), levels of PAH in raw and finished waters are typically much less than the 0.2 µg/l criterion.

Current Levels of Exposure

Section I of this report presents considerable data which may be used to calculate an estimate of human exposure to PAH by all routes of entry to the body. However, quantitative estimates of human exposure to PAH require numerous assumptions concerning principal routes of exposure, extent of absorption, conformity of human lifestyle, and lack of geographic-, sex-, and age-specific variables. Nevertheless, by working with estimates developed for PAH as a class, it is possible through certian extrapolations to arrive at an admittedly crude estimate of PAH exposure.

Unfortunately, there are no environmental monitoring data available for most of the PAH which are specified under the Consent Decree in NRDC v. Train. By for the most widely monitored PAH in the environment is BaP; data on BaP levels in food, air, and water are often used as a measure of total PAH. Among the PAH routinely monitored in water, four compounds are included in the Consent Decree list: BaP, IP, BbFL, and BjFL. In addition, levels of FL and BPR have been routinely determined in water, as recommended by the World Health Organization.

The reported estimated average concentrations of BaP, carcinogenic PAH (BaP, BjFL, and IP), and total PAH in drinking water are 0.55 ng/l, 2.1 ng/l, and 13.5 ng/l, respectively (see Exposure section; Basu and Saxena, 1977-78). Thus, assuming that a human consumes 2 liters of water per day, the daily intake of PAH via drinking water would be:

0.55 ng/l x 2 liters/day = 1.1 ng/day (BaP)

2.1 ng/l x 2 liters/day = 4.2 ng/day (carcinogenic PAH)

13.5 ng/l x 2 liters/day = 27.0 ng/day (total PAH)

Borneff (1977) estimates that the daily dietary intake of PAH is about 8 to 11 µg/day. As a check on this estimate, PAH intake may be calculated based on reported concentrations in various foods (see Exposure section) and the per capita estimates of food consumption by the International Commission on Radiological Protection (1974). Taking a range of 1.0 to 10.0 ppb as a typical concentration for PAH in various foods, and 1,600 g/day as the toal daily food consumption by man from all types of foods (i.e., fruits, vegetables, cereals, dairy products, etc.), the intkae of PAH from the diet would be in the range of 1.6 to 16.0 µg/day. An estimate of BaP ingestion from the diet may be similarly derived. Using 0.1 to 1.0 ppb as the range of BaP concentration in various foods, total daily BaP intake would be .16 to 1.6 µg/day.

Ambient air is reported to contain average levels of 0.5 ng/m^3, 2.0 ng/m^3, and 10.9 ng/m^3 for BaP, carcinogenic PAH, and total PAH, respectively (see Exposure section, Table 16). Taking the range of 15 m^3 to 23 m^3 as the average amount of air inhaled by a human each day results in an estimated intake of 0.005 to 0.0115 ng/day, 0.03 to 0.046 ng/day, and 0.164 to 0.251 ng/day for BaP, carcinogenic PAH, and total PAH, respectively.

In summary, a crude estimate of total daily exposure to PAH would be as follows:

Table 33

Estimate of Human Exposure to PAH from Various Media

Source	Estimated Exposure BaP	Carcinogenic PAH[a]	Total PAH
Water	0.0011 μg/day	0.0042 μg/day	0.027 μg/day
Food	.16-1.6 μg/day		1.6-16. μg/day
Air	0.005-.0115 μg/day	0.03-0.046 μg/day	0.164-0.251 μg/day
Total	.166-1.6 μg/day		1.6-16. μg/day

[a] Total of BaP, BjFL and IP; no data are available for food

Two important factors are not taken into account in this estimate. First, it is known that tobacco smoking can contribute greatly to PAH exposure in man. Exposure to BaP from smoking one pack of cigarettes per day was shown to be 0.4 μg/day (Natl. Acad. Sci. 1972). Second, the possibility for dermal absorption of PAH is assumed to contribute only a negligible amount to the total exposure. Only in certain occupational situations is dermal exposure expected to be quantitatively important.

Special Groups at Risk

An area of considerable uncertainty with regard to the carcinogenic hazard of PAH to man involves the relationship between aryl hydrocarbon hydroxylase (AHH) activity and cancer risk. Genetic variation in AHH inducibility has been implicated as a determining factor for susceptibility to lung and laryngeal cancer (Kellerman, et al. 1973a,b). It was suggested that the extent of AHH inducibility in lymphocytes was correlated with increasing susceptibility to lung cancer formation.

Paigen, et al. (1978) have examined the question of genetic susceptibility to cancer, and concluded that epidemiologic evidence supports this hypothesis. Moreover, they were able to show that AHH inducibility in lymphocytes segregates in the human population as a genetic trait. However, their studies failed to find a correlation between this inducibility and presumed cancer susceptibility, either among healthy relatives of cancer patients or in patients who had their cancer surgically removed. It is noteworthy that previous investigations on AHH inducibility were conducted in persons with active cancer.

Recent studies with other human tissues (liver and placenta) have provided important new data concerning the carcinogen-metabolizing capacity of man and its implications for cancer susceptibility. Conney, et al. (1976) examined individual differences in the metabolism of drugs and carcinogens in human tissues, and have identified drugs which may serve as model substrates to provide an indirect index of carcinogen metabolism for man. The rates for antipyrene, hexobarbital, and zoxazolamine hydroxylation in human autopsy livers were highly, but not perfectly, correlated with the rates of BaP metabolism. In human placenta, an almost perfect correlation was found between zoxazolamine hydroxylase activity and BaP hydroxylase activity. (Kapitulnik, et al. 1976). Thus, metabolism of BaP and zoxazolamine by human placenta occurs by the same enzyme systems(s) or by different enzyme systems under the same regulatory control (Kapitulnik, et al. 1977a). BaP and zoxazolamine hydroxylase activities were also shown to be significantly enhanced in placentas obtained from women who smoked cigarettes.

The lack of perfect correlations for the hepatic metabolism of BaP and certain drugs in many subjects indicated the presence of several monooxygenases in human liver which catalyze the oxidative metabolism of these compounds. Furthermore, large inter-individual differences exist in the capacity of humans to metabolize foreign chemicals both *in vitro* and *in vivo*. Further studies showed that 7,8-benzoflavone markedly stimulated the hydroxylation of BaP, antipyrene, and zoxazolamine in human liver samples, but with a wide variation in magnitude among different samples. These results suggested the presence of multiple monooxygenases or cytochrome P-450 in the different liver samples (Kapitulnik, et al. 1977b). Moreover, 7,8-benzoflavone did not affect the hydroxylation of coumarin or hexobarbital, thereby indicating the existence of different monooxygenases for metabolism of these substrates.

Multiple forms of cytochrome P-450 have been shown in the livers of rats, rabbits, and mice, but not thus far in humans (Kapitulnik, et al. 1977a). More important, however, MCA is a potent inducer of BaP hydroxylase activity in rats but does not stimulate antipyrene hydroxylase, clearly suggesting that metabolism of PAH in rodents may be regulated by different enzyme systems than in humans (Kapitulnik, et al. 1977a).

In contrast to the apparent multiplicity of cytochrome P-450 dependent enzyme systems for the oxidative meatbolism of PAH in man, a single epoxide hydrase with broad substrate specificity may be present in human liver (Conney, et al. 1976; Kapitulnik, et al. 1977c). Because the hydration of arene oxides may lead to the formation of dihydrodiol carcinogen precursors, the capacity of different humans to metabolize epoxides may affect cancer susceptibility. It is not known, however, if enhanced dihydrodiol formation would increase cancer risk or decrease cancer risk.

Thomson and Slaga (1976) did not obtain a correlation of AHH induction with skin-tumor-inducing ability in mice for a series of unsubstituted hydrocarbons. Nevertheless, the highest AHH enzyme activity was found in the epidermal layer of the skin, which is the major point of contact with many environmental chemicals. These results may be interpreted to indicate that a chemical carcinogen may not necessarily induce its own bioactivation, but instead can be transformed into a reactive intermediate by virtue of increased AHH activity stimulated by other noncarcinogenic compounds.

Due consideration must also be given to the fact that, in addition to the initiation of resting cells by a chemical carcinogen, a promotion phase involving cell proliferation is also involved in skin carcinogenesis (Yuspa, et al. 1976). Therefore, although certain aromatic hydrocarbons are effective enzyme inducers, their bioactivated metabolites may function only as an initiator having no promoting ability. A potent complete carcinogen, however, will be transformed not only into a powerful tumor initiator but will also be able to interact with cellular membranes, alter genetic expression, and ultimately cause irreversible cell proliferation. These observations raise certain doubts concerning the validity and/or reliability of equating enzyme inducibility with carcinogenic potential for chemical agents. Further reinforcement of this opinion has been provided by Shulte-Hermann (1977) who showed that cell proliferation is not a direct result of enzyme induction, even though both processes are normally coupled.

The further possibility that the genetics of AHH inducibility is organ-dependent rather than strain-dependent in animals has important implications for evaluating susceptibility to PAH-induced cancers (Kouri, et al. 1976). Most significant is the demonstration that pulmonary AHH may be inducible in all strains of mice, regardless of the inducibility of hepatic AHH. Since the respiratory epithelium represents a primary portal of entry for PAH, AHH activity which is induced in this tissue may bear importantly on susceptibility to malignancy.

Enzyme induction by PAH is not limited to AHH. Owens (1977) recently demonstrated that MCA can induce hepatic UDP-glucuronosyltransferase activity in certain inbred strains of mice. This enzyme catalyzes the conjugation and excretion of PAH substrates after they have first been oxygenated by AHH. The induction of this transferase activity and that of AHH was apparently regulated by a single genetic locus. However, transferase inducibility does not depend on AHH levels, but rather is stoichiometrically related to the concentration of a specific and common cytosolic receptor regulating both enzyme induction processes. Owens further demonstrated that AHH activity can be fully induced in certain mouse strains (e.g., by 2,3,7,8-tetrachlorodibenzo-p-dioxin) without greatly enhancing the transferase activity. Earlier studies had established that chrysene and chlorpromazine were potent inducers of AHH activity while having little effect on transferase activity (Aitio, 1974a,b). Subsequent exposure to carcinogenic PAH (i.e., MCA) could lead to maximal oxidative metabolism but little transferase-catalyzed removal of metabolites by glucuronic acid conjugation. This situation would be exacerbated by the fact that metabolites of MCA are incapable of further inducing the transferase activity. This effect may have considerable toxicologic significance in that highly reactive epoxides of PAH formed by the action of AHH under these circumstances may not be adequately removed by glucuronidation. Thus, one must consider the total exposure of all environmental agents and their possible effect on critical enzymatic processes before attempting to assess the toxicologic impact of exposure to a specific PAH. In summary, there is a need to further explore the relative effects of enzyme induction on the metabolic activation of chemicals to toxic products, versus metabolism of chemicals via detoxification pathways, when considering the possibility of special groups at risk.

Basis and Derivation of Criterion

The presently available data base is inadequate to support the derivation of individual criteria for each of the PAH as specified under the Consent Decree. This problem arises primarily from the diversity of test systems and bioasssay conditions employed for determining carcinogenic potential of individual PAH in experimental animals. Furthermore, it is not possible to estimate the intake via water of individual PAH, except for those compounds which have been selected by the World Health Organization for environmental monitoring. Therefore, an approach to criterion development is adopted in this report with the objective of deriving a single criterion to encompass the entire PAH class. This approach is attractive in that it recognizes the fact that environmental exposures to PAH invariably occur by contact with complex, undefined, PAH mixtures.

The attempt to develop a drinking water criterion for PAH as a class is hindered by several gaps in the scientific data base:

(1) The PAH class is composed of numerous compounds having diverse biological effects and varying carcinogenic potential. A "representative" PAH mixture, has not been defined.

(2) The common practice of using data derived from studies with BaP to make generalizations concerning the effects of environmental PAH may not be scientifically sound.

(3) No chronic animal toxicity studies exist involving oral exposure to PAH mixtures.

(4) No direct human data exist concerning the effects of exposure to defined PAH mixtures.

However, assuming that the development of a criterion must proceed despite these obstacles, certain approaches may be taken to circumvent deficiencies in the data base. The choice of an appropriate animal bioassay from which to derive data for application to the linear non-threshold model for human cancer risk assessment (see Appendix I) should be guided by several considerations. Primary emphasis must be placed on appropriate animal studies which: (1) include sufficient numbers of animals for statistically reliable results; (2) involve long-term low-level exposures to PAH; (3) include a proper control group; and (4) achieve positive dose-related carcinogenic response.

Because there are no studies available regarding chronic oral exposure to PAH mixtures, it is necessary to derive a criterion based upon data involving exposure to a single compound. Even when considering single chemicals, almost no studies are available which involved oral exposure at more than one dose level to a reasonable number of animals. Two studies have been selected, one involving BaP ingestion (Rigdon and Neal, 1967) and one involving DBA ingestion (Snell and Stewart, 1962). Both compounds are recognized as animal carcinogens, and both are known to be environmental contaminants to which humans are exposed.

In the strictest sense it can be argued that a criterion for a chemical class derived from experiments involving a single component of that class is invalid. On the other hand, selection of those components (e.g., BaP and DBA) which are among the more potent carcinogens in the PAH class should lead to a conservative criterion approach. It must be assumed that interactions among the various PAH components resulting in either an enhancement or inhibition of biological effect (see Effects section) will cancel each other out in the environment. Presently, there is no way to quantitate the potential human health risks incurred by the interaction of PAH, either anomg themselves or with other agents (e.g., tumor initiators, promoters, inhibitors) in the environment. In addition, it is known that PAH commonly produce tumors at the site of contact (i.e., forestomach tumors by oral exposure to BaP; lung tumors by intratracheal administration; skin tumors be dermal application). Thus, consideration of the extent of absorption may not always be necessary in the case of carcinogenic PAH, and will in fact result in underestimation of actual risk if only distant target sites are considered. Calculations of water quality criteria for PAH based upon bioassay data for BaP and DBA are presented in Appendix I.

The water quality criteria for BaP and DBA derived using the linear non-threshold model as described in the Appendix are 9.7 ng/l and 43 ng/l, respectively. For the sake of comparison, a water quality criterion for DBA was calculated using the procedure developed by Mantel and Bryan

(1961). As opposed to the linear non-threshold model, which is logistic and defines acceptable risk as 1/100,000, the Mantel and Bryan (1961) model is probablistic and defines acceptable risk as 1/100,000,000. Furthermore, the Mantel and Bryan (1961) is concerned with the maximum tumor incidence in treated animals at the 99 percent assurance level. Using the Mantel and Bryan (1961) approach with DBA, the resultant water quality criterion is 13.3 ng/l.

Under the Consent Decree in NRDC v. Train, criteria are to state "recommended maximum permissible concentrations (including where appropriate, zero) consistent with the protection of aquatic organisms, human health, and recreational activities." BaP and DBA are known animal carcinogens. Because there is no recognized safe concentration for a human carcinogen, the recommended concentration in water for maximum protection of human health is zero.

Because attaining a zero concentration level may be infeasible in cases and in order to assist the Agency and States in the possible future development of water quality regulations, the concentrations of BaP and DBA corresponding to several incremental lifetime cancer risk levels have been estimated. A cancer risk level provides an estimate of the additional incidence of cancer that may be expected in an exposed population. A risk of 10^{-5} for example, indicates a probability of one additional case of cancer for every 100,000 people exposed, a risk of 10^{-6} indicates one additional case of cancer for every million people exposed, and so forth.

PAH are widely distributed in the environment as evidenced by their detection in sediments, soils, air, surface waters, and plant and animal tissues. The ecological impact of these chemicals, however, is uncertain. Numerous studies show that despite their high lipid solubility, PAHs show little tendency for bioaccumulation in the fatty tissues of animals or man. This observation is not unexpected, in light of convincing evidence to show that PAH are rapidly and extensively metabolized.

Lu, et al. (1977) have published the only available study regarding the bioconcentration and biomagnification of a PAH in model ecosystem environments. They reported that the bioconcentration of BaP, expressed as concentration in mosquitofish/concentration in water was zero. This was apparently due to the fact that the fish metabolized the BaP about as rapidly as it was absorbed. On the other hand, in a 33 day terrestrial-aquatic model ecosystem study, BaP showed a small degree of biomagnification which probably resulted from food chain transfer. In this case the biomagnification factor for mosquitofish was 30. Based on the results of Lu, et al. (1977) a bioconcentration (BCF) factor of 30 was employed for the purpose of calculating a water quality criterion. In contrast, as can be noted in Table 6a, the BCF derived from octanol-water partition coefficients for BaP is 6800.

In the Federal Register notice of availability of draft ambient water quality criteria, EPA stated that it is considering setting criteria for BaP and DBA at an interim target risk level of 10^{-5}, 10^{-6} or 10^{-7} as shown in the table below.

BaP

Exposure Assumptions (per day)	Risk Levels and Corresponding Criteria (1) ng/l			
	0	10^{-7}	10^{-6}	10^{-5}
2 liters of drinking water and consumption of 18.7 grams fish and shellfish. (2)	0	0.097	0.97	9.7
Consumption of fish and shellfish only.		0.44	4.45	44.46

DBA

Exposure Assumptions (per day)	Risk Levels and Corresponding Criteria (1) ng/l			
	0	10^{-7}	10^{-6}	10^{-5}
2 liters of drinking water and consumption of 18.7 grams fish and shellfish. (2)	0	0.043	0.43	4.30
Consumption of fish and shellfish only.		0.196	1.96	19.63

(1) Calculated by applying a modified "one hit" extrapolation model described in the FR 15926, 1979. Appropriate bioassay data used in the calculation of the model are presented in Appendix I. Since the extrapolation model is linear to low doses, the additional lifetime risk is directly proportional to the water concentration. Therefore, water concentrations corresponding to other risk levels can be derived by multiplying or dividing one of the risk levels and corresponding water concentrations shown in the table by factors such as 10, 100, 1,000, and so forth.

(2) Approximately 22 percent of the PAH exposure assumed to be BaP, results from the consumption of aquatic organisms which exhibit an average bioconcentration potential of 30 fold. The remaining 78 percent of PAH exposure results from drinking water.

Concentration levels were derived assuming a lifetime exposure to various amounts of PAH (1) occurring from the consumption of both drinking water and aquatic life grown in water containing the corresponding PAH concentrations and, (2) occurring solely from the consumption of aquatic life grown in the waters containing the corresponding PAH concentrations. Because data indicating other sources of exposure and the concentration to total body burden are inadequate for quantitative use, the criterion reflects the increment to risks associated with ambient water exposure only.

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APPENDIX I

Carcinogenicity Risk Assessment by Extrapolation from Laboratory Animal Toxicity Tests

An assessment of health risks associated with exposures of a general environmental nature requires prediction of effects from low level exposures of lifetime duration. Carcinogenic risks effects from environmental exposures must normally be estimated from animal data obtained at much higher levels because of the difficulty in detecting a small increase in tumor induction resulting from long-term low level exposure. Because the carcinogenic process is generally believed to be irreversible, self-replicating, and often originating from a single somatic cell mutation, assumptions of threshold levels of effect are believed to be invalid for many, if not all, cancer-causative compounds. Although many models have been proposed for extrapolation from animal data to human risk assessment, the one utilized here was chosen to facilitate uniform treatment of the variety of chemical compounds that are discussed in the development of those water criterion documents which deal with animal carcinogens.

It is recognized that the process of evaluating existing studies and resultant data in preparation for application of mathematical methods involves a high level of professional judgment. Many questions will necessarily arise due to the unique characteristics of the specific compounds under discussion and the tremendous variability in completeness and comparability among the available studies.

A general explanation of the evaluation and extrapolation procedures to be used are as follows:

1. Since the compounds discussed are known, or suspect, carcinogens, emphasis was placed on those studies with carcinogenic or mutagenic endpoints. In particular, those studies dealing with mammalian species.
2. The extrapolation method employed is a mathematical procedure which uses a single dose and observed response of a toxicological experiment to estimate a dose level for humans that will not increase the risk of tumors by more than a specified level (1 in 100,000) (Personal communication. Dr. Todd Thorsland, CAG, U.S.EPA, Washington, D.C.). Clearly this method is predicated on sound toxicologic test procedures. Hence, each included study was evaluated for adherence to sound toxicological and statistical principles.
3. Judgment was exercised in prioritizing the significance of toxicologic studies that use different routes of administration. In general, the preferred route of exposure is oral (food, water, or gavage) followed by intraperitoneal, intravenous, inhalation, or dermal routes of administration for the same species. However, in some instances consideration of absorption rates required that other routes be evaluated.

The NCI's Ad Hoc Committee on the Evaluation of Low Levels of Environmental Chemical Carcinogens outlined two conditions that would render the extrapolation of animal carcinogenesis to man inappropriate. This committee reported to the Surgeon General as follows:

> "Any substance which is shown conclusively to cause tumors in animals should be considered carcinogenic and, therefore, a potential hazard for man. Exceptions should be considered only where the carcinogenic effect is clearly shown the results from physical rather than chemical induction or where the route of administration is shown to be grossly inappropriate in terms of conceivable human exposure."

4. After selection of the sound toxicologic studies that form the basis for development if a recommended criteria, a single dose and observed response was selected for the most "sensitive" sex (if both males and females were tested) according to the following method: Select the lowest dose which yields a tumor response rate that is greater than the control rate. If the standard controls and media control response rates are not significantly different (<0.05), a combined rate was calculated from controls.

5. The extrapolation methods were applied independently to each selected dose and response pair. The lowest projected dose was selected as the "safe level" based on the available toxicologic studies, if judgement indicated equal confidence in the various dose-response pairs.
6. The calculated safe dose was evaluated along with the results from human studies to delvelop a recommended criteria.

Calculation of Estimated Safe Levels for Humans:

The specific data analyses performed along with required input data are described following in Mathematical Description of Extrapolation Method. This model provides the additional risk associated with ingestion of 2 liters of water per day and contaminated aquatic foods. Any other risks associated with air, food, or other exposure are not addressed by this model. A copy of the working data sheet is also included.

Mathematical Description of Extrapolation Method

A. Necessary information:

Nt = No. of animals (males or females) exposed to selected dose that developed tumors (all sites combined unless tumors appear to be related to route of administration, e.g., peritoneal tumors would not be included if interperitoneal injection method is used).

NT = Total number of animals (male or females) exposed to selected dose level.

nc = Number of control animals (males or females) with tumors.

NC = Total number of control animals (males or females).

Le = Actual maximum lifespan for test animals.

le = Length of exposure (no. of hours, days, weeks, etc.)

d = Average dose per unit of time (mg/kg).

w = Average weight of test animals (kg).

B. Necessary information from general literature:

70 kg = Average weight of man.

L = Theoretical average length of life for test species, unless specified in article. (See attached table for appropriate values)

F = Average weight of fish consumed per day, assumed 18.7 grams.

C. Necessary ecological information:

R = Bioaccumulation factor for edible portions of fish (Supplied by Environmental Research Laboratory, Duluth)

(Note: If a bioacculumation factor is provided for the total fish or for some part other than the total edible portion (such as the fat) an attempt should be made to estimate factor for edible portion).

D. Mathematical Model

$$Pt = Pc + (1-Pc) \left[1 - e^{-t^3 BD}\right]$$

Where:

$Pt = nt \div NT$ = Proportion of test animals with tumors.

$Pc = nc \div NC$ = Proportion of control animals with tumors.

$D = \frac{d \times le}{Le}$ = Lifespan weighted average dose level (mg/kg)/(unit of Time).

$$B = \left\{ \ln \left[\frac{1 - Pt}{1 - Pc}\right] \right\} \div \left[D \times t^3\right]$$

where $t = \frac{\text{lifespan for test animals}}{\text{length of life for species}} = \frac{Le}{L}$

$B' = B\sqrt[3]{\frac{70}{w}}$ (Note: It is assumed that average weight of man = 70 kg.)

If and only if $B' < 0.1$ then

$$SL = \frac{10^{-5} \times 70}{+B'(2 + RxF)} = \text{Safe level (mg/l) for man}$$

If $B' \geq 0.1$ then

$$SL = \frac{\ln(1 - 10^{-5})}{+B'(2 = RxF)} \times 70 = \text{Safe level (mg/l) for man}$$

(Note: It is assumed average daily consumption is 2 liter/day)

APPENDIX II

Summary and Conclusion Regarding the Carcinogenicity of Polynuclear Aromatic Hydrocarbons (PAH)

Polynuclear aromatic hydrocarbons (PAH) comprise a diverse class of compounds consisting of substituted and unsubstituted polycyclic and heterocyclic aromatic rings. They are formed as a result of incomplete combustion of organic compounds and appear in food as well as ambient air and water.

Numerous studies of workers exposed to coal gas, coal tars, and coke oven emissions, all of which have large amounts of PAH, have demonstrated a positive association between the exposures and lung cancer.

Several PAH are well-known animal carcinogens, others are not carcinogenic alone but can enhance or inhibit the response of the carcinogenic PAH and some induce no tumors in experimental animals. Most of the information about the combined carcinogenic effects of several PAH come from skin painting and subcutaneous injection experiments in mice whereas oral administration, intratrachael instillation and inhalation have been shown to induce carcinogenic responses to single compounds. In one subcutaneous injection study in mice it was shown that a combination of several non-carcinogenic PAH compounds, mixed according to the proportion occurring in auto exhaust, does not enhance or inhibit the action of two potent PAH carcinogens, benzo(a)pyrene (BaP) and dibenz(a,h)anthracene.

The mutagenicity of PAH in the Salmonella typhimurium assay correlates well with their carcinogenicity in animal systems. PAH compounds have damaged chromosomes in cytogenetic tests, have induced mutations in mammalian cell culture systems and have induced DNA repair synthesis in human fibroblast cultures.

The water quality criterion for carcinogenic PAH compounds is based on the assumption that each compound is as potent as BaP and that the carcinogenic effect of the compounds is proportional to the sum of their concentrations. Based on an oral feeding study of BaP in mice, the concentration of BaP estimated to result in a lifetime risk of 10^{-5} is 9.7 nanograms per liter. Therefore, with the assumption above, the sum of the concentrations of all carcinogenic PAH compounds should be less than 9.7 nanograms per liter in order to keep the lifetime cancer risk below 10^{-5}.

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Summary of Pertinent Data

The water quality criterion for PAH is based on the experiment reported by Neal and Rigdon (1967) in which benzo(a)-pyrene at doses ranging between 1 and 250 ppm in the diet was fed to strain CFW mice for approximately 110 days. Stomach tumors, which were mostly squamous cell papillomas but some carcinomas, appeared with an incidence statistically higher than controls at doses of 45 ppm and above. At 45 ppm the incidence in controls and treated groups was 0/289 and 4/40, respectively. The one-hit model has the following parameters:

$n_t = 4$
$N_t = 40$
$n_c = 0$
$N_c = 289$
$Le = 110$ days
$le = 110$ days

$d = 45$ ppm X $0.13 = 5.85$ mg/kg/day
$w = 0.034$ kg
$L = 78$ weeks X 7 days/wk = 546 days
$R = 30$
$F = .0187$ kg/day

With these values, the one-hit slope parameter is $B_H = 28.020$ $(\text{mg/kg/day})^{-1}$.

The result is that the water concentration of BaP should be less than 9.7 nanograms per liter in order to keep the individual lifetime risk below 10^{-5}. On the conservative assumptions that all carcinogenic PAH compounds are as potent as BaP, that the effect of a mixture of carcinogenic PAH compounds depends on the sum of their concentrations, and that the non-carcinogenic PAH compounds have no effect on the response of the carcinogenic PAH, it follows that the sum of the concentration of all carcinogenic PAH compounds should be less than 9.7 nanograms per liter in order to keep the lifetime risk less than 10^{-5}.